PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/47, 14/705, 16/18, 16/28, A61K 38/17, 39/395, G01N 33/68

A1

(11) International Publication Number: **WO 99/35166**

(43) International Publication Date: 15 July 1999 (15.07.99)

(21) International Application Number: PCT/CA98/01208

(22) International Filing Date: 30 December 1998 (30.12.98)

(30) Priority Data:
09/001,511 31 December 1997 (31.12.97) US

(71) Applicant *(for all designated States except US)*: ADHEREX TECHNOLOGIES [CA/CA]; 724 Parkdale Avenue, Ottawa, Ontario K1Y 1J6 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BLASCHUK, Orest, W. [CA/CA]; Apartment 1520, 4998 de Maisonneuve West, Westmount, Quebec H3Z 1N2 (CA). GOUR, Barbara, J. [CA/CA]; 5155 Notre Dame de Grace Avenue, Montreal, Quebec H4A 1K6 (CA).

(74) Agents: NASSIF, Omar, A. et al.; Gowling, Strathy & Henderson, Suite 4900, Commerce Court West, Toronto, Ontario M5L 1J3 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: COMPOUNDS AND METHODS FOR MODULATING OCCLUDIN RELATED TISSUE PERMEABILITY

(57) Abstract

Methods for using modulating agents to enhance or inhibit occludin–mediated cell adhesion in a variety of *in vivo* and *in vitro* contexts are provided. Within certain embodiments, the modulating agents may be used to increase vasopermeability. The modulating agents comprise at least one occludin cell adhesion recognition sequence or an antibody or fragment thereof that specifically binds the occludin cell adhesion recognition sequence. Modulating agents may additionally comprise one or more cell adhesion recognition sequences recognized by other adhesion molecules. Such modulating agents may, but need not, be linked to a targeting agent, drug and/or support material.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senégal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

COMPOUNDS AND METHODS FOR MODULATING OCCLUDIN RELATED TISSUE PERMEABILITY

TECHNICAL FIELD

The present invention relates generally to methods for regulating occludin-mediated processes, and more particularly to the use of modulating agents comprising an occludin cell adhesion recognition sequence and/or an antibody that specifically recognizes such a sequence for inhibiting functions such as cell adhesion and the formation of tissue permeability barriers.

BACKGROUND OF THE INVENTION

Cell adhesion is a complex process that is important for maintaining tissue integrity and generating physical and permeability barriers within the body. All tissues are divided into discrete compartments, each of which is composed of a specific cell type that adheres to similar cell types. Such adhesion triggers the formation of intercellular junctions (*i.e.*, readily definable contact sites on the surfaces of adjacent cells that are adhering to one another), also known as tight junctions, gap junctions, spot desmosomes and belt desmosomes. The formation of such junctions gives rise to physical and permeability barriers that restrict the free passage of cells and other biological substances from one tissue compartment to another. For example, the blood vessels of all tissues are composed of endothelial cells. In order for components in the blood to enter a given tissue compartment, they must first pass from the lumen of a blood vessel through the barrier formed by the endothelial cells of that vessel. Similarly, in order for substances to enter the body via the gut, the substances must first pass through a barrier formed by the epithelial cells of that tissue. To enter the blood via the skin, both epithelial and endothelial cell layers must be crossed.

Cell adhesion is mediated by specific cell surface adhesion molecules (CAMs). There are many different families of CAMs, including the immunoglobulin,

integrin, selectin and cadherin superfamilies, and each cell type expresses a unique combination of these molecules. Cadherins are a rapidly expanding family of calcium-dependent CAMs (Munro et al., *In: Cell Adhesion and Invasion in Cancer Metastasis*, P. Brodt, ed., pp. 17-34, RG Landes Co.(Austin TX, 1996). The cadherins (abbreviated CADs) are membrane glycoproteins that generally promote cell adhesion through homophilic interactions (a CAD on the surface of one cell binds to an identical CAD on the surface of another cell). Cadherins have been shown to regulate epithelial, endothelial, neural and cancer cell adhesion, with different CADs expressed on different cell types. For example, N (neural) - cadherin is predominantly expressed by neural cells, endothelial cells and a variety of cancer cell types. E (epithelial) - cadherin is predominantly expressed by epithelial cells. VE (vascular endothelial) - cadherin is predominantly expressed by endothelial cells. Other CADs are P (placental) - cadherin, which is found in human skin, and R (retinal) - cadherin. A detailed discussion of the cadherins is provided in Munro SB et al., 1996, *In: Cell Adhesion and Invasion in Cancer Metastasis*, P. Brodt, ed., pp.17-34 (RG Landes Company, Austin TX) and Lampugnani and Dejana, *Curr. Opin. Cell Biol. 9:674-682*, 1997.

CAD-mediated cell adhesion triggers a cascade of events that lead to the formation of intercellular junctions, and ultimately to the establishment of permeability barriers between tissue compartments. The intercellular junction that is directly responsible for the creation of permeability barriers that prevent the diffusion of solutes through paracellular spaces is known as the tight junction, or zonula occludens (Anderson and van Itallie, *Am. J. Physiol. 269:G467-G475*, 1995; Lampugnani and Dejana, *Curr. Opin. Cell Biol. 9:674-682*, 1997).

Occludin is a transmembrane component of tight junctions (Furuse et al., *J. Cell Biol. 123:1777-1788*, 1993; Furuse et al., *J. Cell Sci. 109:429-435*, 1996). This protein appears to be expressed by all endothelial cell types, as well as by most epithelial cell types. Occludin is an integral membrane protein (Figure 1) that is composed of two extracellular domains (EC1 and EC2), four hydrophobic domains (TM1-TM4) that transverse the plasma membrane, and three cytoplasmic domains (CP1-CP3). The structures of all known mammalian occludins are similar (Figure 2;

Ando-Akatsuka et al., *J. Biol. Chem. 133*:43-47, 1996). Occludin is believed to be directly involved in cell adhesion and the formation of tight junctions (Furuse et al., *J. Cell Sci. 109*:429-435, 1996; Chen et al., *J. Cell Biol. 138*:891-899, 1997). It has been proposed that occludin promotes cell adhesion through homophilic interactions (an occludin on the surface of one cell binds to an identical occludin on the surface of another cell). A detailed discussion of occludin structure and function is provided by Lampugnani and Dejana, *Curr. Opin. Cell Biol. 9:674-682*, 1997.

Although cell adhesion is required for certain normal physiological functions, there are situations in which the level of cell adhesion is undesirable. For example, many pathologies (such as autoimmune diseases and inflammatory diseases) involve abnormal cellular adhesion. Cell adhesion may also play a role in graft rejection. In such circumstances, modulation of cell adhesion may be desirable.

In addition, permeability barriers arising from cell adhesion create difficulties for the delivery of drugs to specific tissues and tumors within the body. For example, skin patches are a convenient tool for administering drugs through the skin. However, the use of skin patches has been limited to small, hydrophobic molecules because of the epithelial and endothelial cell barriers. Similarly, endothelial cells render the blood capillaries largely impermeable to drugs, and the blood/brain barrier has hampered the targeting of drugs to the central nervous system. In addition, many solid tumors develop internal barriers that limit the delivery of anti-tumor drugs and antibodies to inner cells.

Attempts to facilitate the passage of drugs across such barriers generally rely on specific receptors or carrier proteins that transport molecules across barriers *in vivo*. However, such methods are often inefficient, due to low endogenous transport rates or to the poor functioning of a carrier protein with drugs. While improved efficiency has been achieved using a variety of chemical agents that disrupt cell adhesion, such agents are typically associated with undesirable side-effects, may require invasive procedures for administration and may result in irreversible effects.

Accordingly, there is a need in the art for compounds that modulate cell adhesion and improve drug delivery across permeability barriers without such

disadvantages. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention provides compounds and methods for modulating occludin-mediated cell adhesion and the formation of permeability barriers. Within certain aspects, compounds provided herein comprise an occludin CAR sequence, or variant thereof that retains the ability to modulate occludin-mediated cell adhesion. Certain compounds are cyclic peptides that comprise the sequence LYHY (SEQ ID NO:1). Within certain embodiments, such cyclic peptides have the formula:

$$(Z_1)\text{-}(Y_1)\text{-}(X_1)\text{-Leu-Tyr-His-Tyr-}(X_2)\text{-}(Y_2)\text{-}(Z_2)$$

wherein X_1, and X_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds, and wherein X_1 and X_2 independently range in size from 0 to 10 residues, such that the sum of residues contained within X_1 and X_2 ranges from 1 to 12; wherein Y_1 and Y_2 are independently selected from the group consisting of amino acid residues, and wherein a covalent bond is formed between residues Y_1 and Y_2; and wherein Z_1 and Z_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds. Such cyclic peptides may comprise modifications such as an N-acetyl or N-alkoxybenzyl group and/or a C-terminal amide or ester group. Cyclic peptides may be cyclized via, for example, a disulfide bond; an amide bond between terminal functional groups, between residue side-chains or between one terminal functional group and one residue side chain; a thioether bond or $\delta_1\delta_1$-ditryptophan, or a derivative thereof.

Within other embodiments, such compounds may be linear peptides comprising the sequence LYHY (SEQ ID NO:1) or a variant thereof. Such peptides are preferably 4-30 amino acid residues in length, preferably 5-16 amino acid residues, and

more preferably 6-9 amino acid residues.

Within further aspects, the present invention provides cell adhesion modulating agents that comprise a cyclic or linear peptide as described above. Within specific embodiments, such modulating agents may be linked to one or more of a targeting agent, a drug, a solid support or support molecule, or a detectable marker. Within further specific embodiments, cell adhesion modulating agents are provided that comprise a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

Within further related aspects, cell adhesion modulating agents are provided which comprise an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an occludin.

In addition, any of the above cell adhesion modulating agents may further comprise one or more of: (a) a cell adhesion recognition sequence that is bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or (b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

The present invention further provides pharmaceutical compositions comprising a cell adhesion modulating agent as described above, in combination with a pharmaceutically acceptable carrier. Such compositions may further comprise a drug. In addition, or alternatively, such compositions may further comprise one or more of: (a) a peptide comprising a cell adhesion recognition sequence that is bound by an adhesion molecule other than an occludin; and/or (b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

Within further aspects, methods are provided for modulating cell adhesion, comprising contacting a cadherin-expressing cell with a cell adhesion

modulating agent as described above.

Within one such aspect, the present invention provides methods for increasing vasopermeability in a mammal, comprising administering to a mammal a cell adhesion modulating agent as provided above, wherein the modulating agent inhibits occludin-mediated cell adhesion.

Within another aspect, methods are provided for reducing unwanted cellular adhesion in a mammal, comprising administering to a mammal a cell adhesion modulating agent as provided above, wherein the modulating agent inhibits occludin-mediated cell adhesion.

In yet another aspect, the present invention provides methods for enhancing the delivery of a drug through the skin of a mammal, comprising contacting epithelial cells of a mammal with a cell adhesion modulating agent as provided above and a drug, wherein the modulating agent inhibits occludin-mediated cell adhesion, and wherein the step of contacting is performed under conditions and for a time sufficient to allow passage of the drug across the epithelial cells.

The present invention further provides methods for enhancing the delivery of a drug to a tumor in a mammal, comprising administering to a mammal a cell adhesion modulating agent as provided above and a drug, wherein the modulating agent inhibits occludin-mediated cell adhesion.

Within further aspects, the present invention provides methods for treating cancer in a mammal, comprising administering to a mammal a cell adhesion modulating agent as provided above, wherein the modulating agent inhibits occludin-mediated cell adhesion.

The present invention further provides methods for inhibiting angiogenesis in a mammal, comprising administering to a mammal a cell adhesion modulating agent as provided above, wherein the modulating agent inhibits occludin-mediated cell adhesion.

Within further aspects, the present invention provides methods for enhancing drug delivery to the central nervous system of a mammal, comprising administering to a mammal a cell adhesion modulating agent as provided above,

wherein the modulating agent inhibits occludin-mediated cell adhesion.

Within further aspects, methods are provided for modulating the immune system of a mammal, comprising administering to a mammal a modulating agent as described above, wherein the modulating agent inhibits occludin-mediated function.

The present invention further provides methods for modulating the formation of epithelial cell tight junctions, comprising administering to a mammal a cell adhesion modulating agent that comprises the sequence LYHY (SEQ ID NO:1). Within certain embodiments, such modulating agents may stimulate the formation of epithelial cell tight junctions.

Within other aspects, the present invention provides methods for inhibiting the development of diarrhea in a patient, comprising administering to a patient a cell adhesion modulating agent that comprises the sequence LYHY (SEQ ID NO:1), wherein the modulating agent stimulates epithelial cell adhesion.

The present invention further provides methods for enhancing wound healing in a mammal, comprising contacting a wound in a mammal with a cell adhesion modulating agent as provided above, wherein the modulating agent enhances occludin-mediated cell adhesion.

Within a related aspect, the present invention provides methods for enhancing adhesion of foreign tissue implanted within a mammal, comprising contacting a site of implantation of foreign tissue in a mammal with a cell adhesion modulating agent as provided above, wherein the modulating agent enhances occludin-mediated cell adhesion.

The present invention further provides methods for inducing apoptosis in an occludin-expressing cell, comprising contacting an occludin-expressing cell with a cell adhesion modulating agent as provided above, wherein the modulating agent inhibits occludin-mediated cell adhesion.

The present invention further provides methods for identifying an agent capable of modulating occludin-mediated cell adhesion. One such method comprises the steps of (a) culturing cells that express an occludin in the presence and absence of a candidate agent, under conditions and for a time sufficient to allow cell adhesion; and

(b) visually evaluating the extent of cell adhesion among the cells.

Within another embodiment, such methods may comprise the steps of: (a) culturing normal rat kidney cells in the presence and absence of a candidate agent, under conditions and for a time sufficient to allow cell adhesion; and (b) comparing the level of cell surface occludin and E-cadherin for cells cultured in the presence of candidate agent to the level for cells cultured in the absence of candidate agent.

Within a further embodiment, such methods may comprise the steps of: (a) culturing human aortic endothelial cells in the presence and absence of a candidate agent, under conditions and for a time sufficient to allow cell adhesion; and (b) comparing the level of cell surface occludin and N-cadherin for cells cultured in the presence of candidate agent to the level for cells cultured in the absence of candidate agent.

Within yet another embodiment, such methods comprise the steps of: (a) contacting an antibody that binds to a modulating agent comprising the sequence LYHY (SEQ ID NO:1) with a test compound; and (b) detecting the level of antibody that binds to the test compound.

The present invention further provides methods for detecting the presence of occludin-expressing cells in a sample, comprising: (a) contacting a sample with an antibody that binds to an occludin comprising the sequence LYHY (SEQ ID NO:1) under conditions and for a time sufficient to allow formation of an antibody-occludin complex; and (b) detecting the level of antibody-occludin complex, and therefrom detecting the presence of occludin-expressing cells in the sample.

Within further aspects, the present invention provides kits for detecting the presence of occludin-expressing cells in a sample, comprising: (a) an antibody that binds to a modulating agent comprising the sequence LYHY (SEQ ID NO:1); and (b) a detection reagent.

The present invention further provides, within other aspects, kits for enhancing transdermal drug delivery, comprising: (a) a skin patch; and (b) a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein the modulating agent inhibits occludin-mediated

cell adhesion.

These and other aspects of the invention will become evident upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each were individually noted for incorporation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram depicting the structure of a human occludin. The two extracellular domains are designated EC1 and EC2, the four hydrophobic domains that transverse the plasma membrane are represented by TM1-TM4, and the three cytoplasmic domains are denoted CP1-CP3. The occludin cell adhesion recognition sequence, LYHY (Leu-Tyr-His-Tyr; SEQ ID NO:1), along with flanking amino acid residues is shown within EC2 and is indicated by •.

Figure 2 provides the amino acid sequences of mammalian occludin EC2 domains: human (SEQ ID NO:5), mouse (SEQ ID NO:6), dog (SEQ ID NO:7), and rat-kangaroo (SEQ ID NO:8), as indicated, along with the consensus sequence obtained using a Clustal W protein sequence alignment. The occludin cell adhesion recognition sequence, LYHY (Leu-Tyr-His-Tyr; SEQ ID NO:1), along with flanking amino acid residues is shown in bold.

Figures 3A-3D provide the structures of representative cyclic peptide modulating agents.

Figures 4A and 4B are immunofluorescence photographs of monolayer cultures of human aortic endothelial cells immunolabeled for occludin (red color) and VE-cadherin (green color). Colocalization of occludin and VE-cadherin is indicated by the yellow color. Arrows indicate gaps between the cells. The cells were either not treated (Figure 4A), or exposed for 1 hour to 100 μg/ml H-QYLYHYCVVD-OH (SEQ ID NO:2; Figure 4B).

Figure 5 is a photograph of the shaved back of a rat that received duplicate subdermal injections of either phosphate buffered saline, phosphate buffered saline containing acetyl-QYLYHYCVVD-NH_2 (SEQ ID NO:2; Peptide 1) H-

QYLYHYCVVD-NH_2 (SEQ ID NO:2; Peptide 2), or H-QYLYHYCVVD-OH (SEQ ID NO:2; Peptide 3) at a concentration of 100 μg/ml, followed 15 minutes later by a single injection of Evans blue into the tail vein. The photograph was taken 15 minutes after injection of the dye.

Figure 6 is a histogram depicting the optical densities of dimethylformamide extracts prepared from the excised injection sites shown in Figure 5, and showing that more dye was extracted from the sites injected with H-QYLYHYCVVD-OH (SEQ ID NO:2; Peptide 3), than from sites injected with either phosphate buffered saline, acetyl-QYLYHYCVVD-NH_2 (SEQ ID NO:2; Peptide 1) or H-QYLYHYCVVD-NH_2 (SEQ ID NO:2; Peptide 2).

Figure 7 is a photograph of the shaved back of a rat that received duplicate subdermal injections of either phosphate buffered saline, phosphate buffered saline containing acetyl-CLYHYC-NH_2 (SEQ ID NO:3; Peptide 4), or H-CLYHYC-OH (SEQ ID NO:3; Peptide 5) at a concentration of 100 μg/ml, followed 15 minutes later by a single injection of Evans blue into the tail vein. The photograph was taken 15 minutes after injection of the dye.

Figure 8 is a histogram depicting the optical densities of dimethylformamide extracts prepared from the excised sites of the shaved back of a rat that received duplicate subdermal injections of either phosphate buffered saline, phosphate buffered saline containing acetyl-CLYHYC-NH_2 (SEQ ID NO:3; Peptide 4), or H-CLYHYC-OH (SEQ ID NO:3; Peptide 5) at a concentration of 100 μg/ml, followed 15 minutes later by a single injection of Evans blue into the tail vein.

Figure 9 is a histogram depicting the mean electrical resistance across MDCK cell monolayers cultured for 24 hours in medium alone (Control), or medium containing H-QYLYHYCVVD-NH_2 (Peptide 2), H-QYLYHYCVVD-COOH (Peptide 3) or N-Ac-CLYHYC-NH_2 (Peptide 4) at a concentration of 0.5 mg/ml. Duplicate measurements were taken, and error bars represent the standard deviation.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention provides cell adhesion modulating

agents comprising peptides that are capable of modulating occludin-mediated processes, such as cell adhesion. In general, to modulate occludin-mediated cell adhesion, an occludin-expressing cell is contacted with a cell adhesion modulating agent (also referred to herein as a "modulating agent") either *in vivo* or *in vitro*. It has been found, within the context of the present invention, that the second extracellular domain (EC2) of occludin contains a CAR sequence that promotes the formation of permeability barriers. Accordingly, a modulating agent may comprise at least one peptide (which may, but need not, be cyclic) that contains an occludin cell adhesion recognition (CAR) sequence and/or an antibody or fragment thereof that specifically binds to an occludin CAR sequence. In humans and certain other mammals, the CAR sequence is LYHY (Leu-Tyr-His-Tyr; SEQ ID NO:1; see Figure 2 and SEQ ID NOs:5-8). However, the present invention further contemplates occludin CAR sequences from other organisms. Such CAR sequences may be identified based upon sequence similarity to the sequences provided herein, and the ability to modulate an occludin-mediated function may be confirmed as described herein. A modulating agent may further comprise one or more additional CAR sequences and/or antibodies (or antigen-binding fragments thereof) that specifically bind to an occludin CAR sequence. Alternatively, or in addition, a modulating agent may further comprise one or more CAR sequences for a CAM other than an occludin and/or an antibody or antigen-binding fragment thereof that specifically binds to such a CAM.

Certain modulating agents described herein inhibit cell adhesion. Such modulating agents may generally be used, for example, to treat diseases or other conditions characterized by undesirable cell adhesion or to facilitate drug delivery to a specific tissue or tumor. Within other aspects of the present invention, certain modulating agents may be used to enhance cell adhesion (*e.g.*, to supplement or replace stitches or to facilitate wound healing). Certain modulating agents provided herein have the ability to stimulate the formation of tight junctions in epithelial cells, but not in endothelial cells. Such agents may be used, for example, for treating diarrhea.

CELL ADHESION MODULATING AGENTS

The term "cell adhesion modulating agent," as used herein, refers to a molecule comprising at least one of the following components:

(a) a linear or cyclic peptide sequence that is at least 50% identical to an occludin CAR sequence (*i.e.*, an occludin CAR sequence or an analogue thereof that retains at least 50% sequence identity);

(b) a mimetic (*e.g.*, peptidomimetic or small molecule mimic) of an occludin CAR sequence;

(c) a substance, such as an antibody or antigen-binding fragment thereof, that specifically binds an occludin CAR sequence; and/or

(d) a polynucleotide encoding a polypeptide that comprises an occludin CAR sequence or analogue thereof.

A modulating agent may consist entirely of one or more of the above elements, or may additionally comprise further peptide and/or non-peptide regions. Additional peptide or polynucleotide regions may be derived from occludin (preferably an extracellular domain that comprises a CAR sequence) and/or may be heterologous. Certain modulating agents comprise the occludin CAR sequence LYHY (SEQ ID NO:1) or an analogue thereof. Within certain preferred embodiments, such a modulating agent contains 4-30 consecutive amino acid residues, preferably 5-16 consecutive amino acid residues and more preferably 6-9 consecutive amino acid residues, present within an occludin.

An "occludin CAR sequence," as used herein, refers to an amino acid sequence that is present within in a naturally occurring occludin and that is capable of detectably modulating an occludin-mediated function, such as cell adhesion, as described herein. In other words, contacting an occludin-expressing cell with a peptide comprising a CAR sequence results in a detectable change in an occludin-mediated function using at least one of the representative assays provided herein. CAR sequences may be of any length, but generally comprise 4-16 amino acid residues, and preferably 5-8 amino acid residues. As noted above, the four amino acid sequence LYHY (SEQ ID NO:1) is an occludin CAR sequence.

As an alternative to comprising a native occludin CAR sequence,

modulating agents as described herein may comprise an analogue or mimetic of an occludin CAR sequence. Within the specific embodiments described herein, it should be understood that an analogue or mimetic may be substituted for a native CAR sequence within any modulating agent. An analogue generally retains at least 50% identity to a native occludin CAR sequence, and modulates an occludin-mediated function as described herein. Such analogues preferably contain at least three residues of, and more preferably at least five residues of, an occludin CAR sequence. An analogue may contain any of a variety of amino acid substitutions, additions, deletions and/or modifications (*e.g.*, side chain modifications). Preferred amino acid substitutions are conservative. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. The critical determining feature of an occludin CAR sequence analogue is the ability to modulate an occludin-mediated function, which may be evaluated using the representative assays provided herein.

A mimetic is a non-peptidyl compound that is conformationally similar to an occludin CAR sequence, such that it modulates an occludin-mediated function as described below. Such mimetics may be designed based on techniques that evaluate the three dimensional structure of the peptide. For example, Nuclear Magnetic Resonance spectroscopy (NMR) and computational techniques may be used to determine the

conformation of an occludin CAR sequence. NMR is widely used for structural analyses of both peptidyl and non-peptidyl compounds. Nuclear Overhauser Enhancements (NOE's), coupling constants and chemical shifts depend on the conformation of a compound. NOE data provides the interproton distance between protons through space and can be used to calculate the lowest energy conformation for the occludin CAR sequence. This information can then be used to design mimetics of the preferred conformation. Linear peptides in solution exist in many conformations. By using conformational restriction techniques it is possible to fix the peptide in the active conformation. Conformational restriction can be achieved by i) introduction of an alkyl group such as a methyl which sterically restricts free bond rotation; ii) introduction of unsaturation which fixes the relative positions of the terminal and geminal substituents; and/or iii) cyclization, which fixes the relative positions of the sidechains. Mimetics may be synthesized where one or more of the amide linkages has been replaced by isosteres, substituents or groups which have the same size or volume such as $-CH_2NH-$, -CSNH-, $-CH_2S-$, -CH=CH-, $-CH_2CH_2-$, -CONMe- and others. These backbone amide linkages can also be part of a ring structure (*e.g.*, lactam). Mimetics may be designed where one or more of the side chain functionalities of the occludin CAR sequence are replaced by groups that do not necessarily have the same size or volume, but have similar chemical and/or physical properties which produce similar biological responses. Other mimetics may be small molecule mimics, which may be readily identified from small molecule libraries, based on the three-dimensional structure of the CAR sequence. It should be understood that, within embodiments described below, an analogue or mimetic may be substituted for an occludin CAR sequence.

A portion of a modulating agent that comprises an occludin CAR sequence, or analogue or mimetic thereof, may be a linear or cyclic peptide. The term "cyclic peptide," as used herein, refers to a peptide or salt thereof that comprises (1) an intramolecular covalent bond between two non-adjacent residues and (2) at least one occludin CAR sequence. The intramolecular bond may be a backbone to backbone, side-chain to backbone or side-chain to side-chain bond (*i.e.*, terminal functional groups

of a linear peptide and/or side chain functional groups of a terminal or interior residue may be linked to achieve cyclization). Preferred intramolecular bonds include, but are not limited to, disulfide, amide and thioether bonds.

In addition to one or more of the above components, a modulating agent may comprise one or more additional CAR sequences, which may or may not be occludin CAR sequences, and/or one or more antibodies or fragments thereof that specifically recognize a CAR sequence. Additional CAR sequences may be present within a cyclic peptide containing an occludin CAR sequence, within a separate cyclic peptide component of the modulating agent and/or in a non-cyclic portion of the modulating agent. Antibodies and antigen-binding fragments thereof are typically present in a non-cyclic portion of the modulating agent.

Within certain embodiments in which inhibition of cell adhesion is desired, a modulating agent may contain one occludin CAR sequence or analogue thereof. Alternatively, such an agent may comprise multiple occludin CAR sequences, which may be adjacent to one another (*i.e.*, without intervening sequences) or in close proximity (*i.e.*, separated by peptide and/or non-peptide linkers to give a distance between the CAR sequences that ranges from about 0.1 to 400 nm). For example, a modulating agent with adjacent LYHY sequences may comprise the peptide LYHYLYHY (SEQ ID NO:9). A representative modulating agent with LYHY sequences in close proximity may comprise the sequence QLYHYQLYHYQLYHY (SEQ ID NO:10). One or more antibodies, or fragments thereof, may similarly be used within such embodiments, either alone or in combination with one or more CAR sequences.

In certain embodiments, a modulating agent as described above may enhance cell adhesion among epithelial cells, but not among endothelial cells. It has been found, within the context of the present invention, that certain modulating agents comprising an LYHY sequence affect endothelial and epithelial cells differently, stimulating the formation of tight junctions in epithelial cells. Such agents include H-QYLYHYCVVD-COOH and N-Ac-CLYHYC-NH_2. Terminal functional groups may influence the activity of peptide modulating agents in epithelial and endothelial cells.

Within other embodiments in which enhancement of cell adhesion is desired, a modulating agent may generally contain multiple occludin CAR sequences and/or antibodies that specifically bind to such sequences, joined by linkers as described above. Enhancement of cell adhesion may also be achieved by attachment of multiple modulating agents to a support molecule or material, as discussed further below.

A modulating agent as described herein may additionally comprise a CAR sequence for one or more different adhesion molecules (including, but not limited to, other CAMs) and/or one or more antibodies or fragments thereof that bind to such sequences. Linkers may, but need not, be used to separate such CAR sequence(s) and/or antibody sequence(s) from the LYHY sequence(s) and/or each other. Such modulating agents may generally be used within methods in which it is desirable to simultaneously disrupt cell adhesion mediated by multiple adhesion molecules. As used herein, an "adhesion molecule" is any molecule that mediates cell adhesion via a receptor on the cell's surface. Adhesion molecules include classical cadherins; atypical cadherins such as cadherin-11 (OB cadherin), cadherin-5 (VE-cadherin), cadherin-6 (K-cadherin), cadherin-7, cadherin-8, cadherin-12 (Br-cadherin, cadherin-14, cadherin-15, and PB-cadherin; other nonclassical cadherins such as desmocollins (dsc) and desmogleins (dsg); claudin; integrins; and members of the immunoglobulin supergene family, such as N-CAM and PECAM). Preferred CAR sequences for inclusion within a modulating agent include: (a) His-Ala-Val (HAV), which is bound by classical cadherins; (b) Arg-Gly-Asp (RGD), which is bound by integrins (*see* Cardarelli et al., *J. Biol. Chem. 267*:23159-23164, 1992); (c) KYSFNYDGSE (SEQ ID NO:11), which is bound by N-CAM; (d) claudin CAR sequences comprising at least four consecutive amino acids present within a claudin region that has the formula: Trp-Lys/Arg-Aaa-Baa-Ser/Ala-Tyr/Phe-Caa-Gly (SEQ ID NO:47), wherein Aaa, Baa and Caa indicate independently selected amino acid residues; Lys/Arg is an amino acid that is lysine or arginine; Ser/Ala is an amino acid that is serine or alanine; and Tyr/Phe is an amino acid that is tyrosine or phenylalanine; and (e) nonclassical cadherin CAR sequences comprising at least three consecutive amino acids present within a nonclassical cadherin region that has the formula: Aaa-Phe-Baa-Ile/Leu/Val-Asp/Asn/Glu-Caa-Daa-

Ser/Thr/Asn-Gly (SEQ ID NO:48), wherein Aaa, Baa, Caa and Daa are independently selected amino acid residues; Ile/Leu/Val is an amino acid that is selected from the group consisting of isoleucine, leucine and valine, Asp/Asn/Glu is an amino acid that is selected from the group consisting of aspartate, asparagine and glutamate; and Ser/Thr/Asn is an amino acid that is selected from the group consisting of serine, threonine or asparagine. Representative claudin CAR sequences include IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52). Representative nonclassical cadherin CAR sequences include the VE-cadherin CAR sequence DAE; the OB-cadherin CAR sequences DDK, EEY and EAQ; the dsg CAR sequences NQK, NRN and NKD and the dsc CAR sequences EKD and ERD.

A linker may be any molecule (including peptide and/or non-peptide sequences as well as single amino acids or other molecules), that does not contain a CAR sequence and that can be covalently linked to at least two peptide sequences. Using a linker, LYHY (SEQ ID NO:1)-containing peptides and other peptide or protein sequences may be joined head-to-tail (*i.e.*, the linker may be covalently attached to the carboxyl or amino group of each peptide sequence), head-to-side chain and/or tail-to-side chain. Modulating agents comprising one or more linkers may form linear or branched structures. Within one embodiment, modulating agents having a branched structure comprise three different CAR sequences, such as RGD, LYHY (SEQ ID NO:1) and HAV. Within another embodiment, modulating agents having a branched structure may comprise, for example, LYHY (SEQ ID NO:1), along with one or more of a claudin CAR sequence; a VE-cadherin CAR sequence; a dsg CAR sequence and/or a dsc CAR sequence. Linkers preferably produce a distance between CAR sequences between 0.1 to 10,000 nm, more preferably about 0.1-400 nm. A separation distance between recognition sites may generally be determined according to the desired function of the modulating agent. For inhibitors of cell adhesion, the linker distance should be small (0.1-400 nm). For enhancers of cell adhesion, the linker distance should be 400-10,000 nm. One linker that can be used for such purposes is $(H_2N(CH_2)_nCO_2H)_m$, or derivatives thereof, where n ranges from 1 to 10 and m ranges from 1 to 4000. For example, if glycine ($H_2NCH_2CO_2H$) or a multimer thereof is used

as a linker, each glycine unit corresponds to a linking distance of 2.45 angstroms, or 0.245 nm, as determined by calculation of its lowest energy conformation when linked to other amino acids using molecular modeling techniques. Similarly, aminopropanoic acid corresponds to a linking distance of 3.73 angstroms, aminobutanoic acid to 4.96 angstroms, aminopentanoic acid to 6.30 angstroms and amino hexanoic acid to 6.12 angstroms. Other linkers that may be used will be apparent to those of ordinary skill in the art and include, for example, linkers based on repeat units of 2,3-diaminopropanoic acid, lysine and/or ornithine. 2,3-Diaminopropanoic acid can provide a linking distance of either 2.51 or 3.11 angstroms depending on whether the side-chain amino or terminal amino is used in the linkage. Similarly, lysine can provide linking distances of either 2.44 or 6.95 angstroms and ornithine 2.44 or 5.61 angstroms. Peptide and non-peptide linkers may generally be incorporated into a modulating agent using any appropriate method known in the art.

The total number of CAR sequences (including occludin CAR sequence(s), with or without other CAR sequences derived from one or more adhesion molecules) present within a modulating agent may range from 1 to a large number, such as 100, preferably from 1 to 10, and more preferably from 1 to 5. Peptide modulating agents comprising multiple CAR sequences typically contain from 4 to about 1000 amino acid residues, preferably from 4 to 50 residues. When non-peptide linkers are employed, each CAR sequence of the modulating agent is present within a peptide that generally ranges in size from 4 to 50 residues in length, preferably from 4 to 25 residues, more preferably from 4 to 16 residues and still more preferably from 4 to 15 residues. Additional residue(s) that may be present on the N-terminal and/or C-terminal side of a CAR sequence may be derived from sequences that flank the LYHY sequence within naturally occurring occludins with or without amino acid substitutions and/or other modifications. Flanking sequences for mammalian occludins are shown in Figure 2, and in SEQ ID NOs:5-8. Alternatively, additional residues present on one or both sides of the CAR sequence(s) may be unrelated to an endogenous sequence (*e.g.*, residues that facilitate purification or other manipulation and/or residues having a targeting or other function).

A modulating agent may contain sequences that flank the occludin CAR sequence on one or both sides, to enhance potency or specificity. A suitable flanking sequence for enhancing potency includes, but is not limited to, an endogenous sequence present in an occludin (shown in, for example, Figure 2).

To facilitate the preparation of modulating agents having a desired potency, nuclear magnetic resonance (NMR) and computational techniques may be used to determine the conformation of a peptide that confers a known potency. NMR is widely used for structural analysis of molecules. Cross-peak intensities in nuclear Overhauser enhancement (NOE) spectra, coupling constants and chemical shifts depend on the conformation of a compound. NOE data provide the interproton distance between protons through space. This information may be used to facilitate calculation of the lowest energy conformation for the LYHY (SEQ ID NO:1) sequence. Conformation may then be correlated with tissue specificity to permit the identification of peptides that are similarly tissue specific or have enhanced tissue specificity.

Modulating agents may be polypeptides or salts thereof, containing only amino acid residues linked by peptide bonds, or may contain non-peptide regions, such as linkers. Peptide regions of a modulating agent may comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be from natural or non-natural sources, provided that at least one amino group and at least one carboxyl group are present in the molecule; α- and β-amino acids are generally preferred. The 20 L-amino acids commonly found in proteins are identified herein by the conventional three-letter or one-letter abbreviations indicated in Table 1, and the corresponding D-amino acids are designated by a lower case one letter symbol.

Table 1

Amino acid one-letter and three-letter abbreviations

A	Ala	Alanine
R	Arg	Arginine
D	Asp	Aspartic acid
N	Asn	Asparagine

C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

A modulating agent may also contain rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (*e.g.*, benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (*e.g.*, acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (*e.g.*, methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Preferred derivatives include amino acids having a C-terminal amide group. Residues other than common amino acids that may be present with a modulating agent include, but are not limited to, 2-mercaptoaniline, 2-mercaptoproline, ornithine, diaminobutyric acid, α-aminoadipic acid, m-aminomethylbenzoic acid and α,β-diaminopropionic acid.

Certain preferred modulating agents for use within the present invention comprise at least one of the following sequences: QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), and/or LYHY (SEQ ID NO:1), wherein each

amino acid residue may, but need not, be modified as described above. Within other embodiments, a modulating agent may comprise a cyclic peptide of one of the following sequences: CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44) or KLYHYD (SEQ ID NO:45). Modulating agents comprising derivatives of any of the sequences recited herein (*i.e.*, sequences having one or more C-terminal, N-terminal and/or side chain modifications) are also encompassed by the present invention.

Peptide modulating agents (and peptide portions of modulating agents) as described herein may be synthesized by methods well known in the art, including chemical synthesis and recombinant DNA methods. For modulating agents up to about 50 residues in length, chemical synthesis may be performed using standard solution or solid phase peptide synthesis techniques, in which a peptide linkage occurs through the direct condensation of the α-amino group of one amino acid with the α-carboxy group of the other amino acid with the elimination of a water molecule. Peptide bond synthesis by direct condensation, as formulated above, requires suppression of the reactive character of the amino group of the first and of the carboxyl group of the second amino acid. The masking substituents must permit their ready removal, without inducing breakdown of the labile peptide molecule.

In solution phase synthesis, a wide variety of coupling methods and protecting groups may be used (*see* Gross and Meienhofer, eds., "The Peptides: Analysis, Synthesis, Biology," Vol. 1-4 (Academic Press, 1979); Bodansky and Bodansky, "The Practice of Peptide Synthesis," 2d ed. (Springer Verlag, 1994)). In addition, intermediate purification and linear scale up are possible. Those of ordinary skill in the art will appreciate that solution synthesis requires consideration of main chain and side chain protecting groups and activation method. In addition, careful segment selection is necessary to minimize racemization during segment condensation. Solubility considerations are also a factor.

Solid phase peptide synthesis uses an insoluble polymer for support during organic synthesis. The polymer-supported peptide chain permits the use of

simple washing and filtration steps instead of laborious purifications at intermediate steps. Solid-phase peptide synthesis may generally be performed according to the method of Merrifield et al., *J. Am. Chem. Soc.* 85:2149, 1963, which involves assembling a linear peptide chain on a resin support using protected amino acids. Solid phase peptide synthesis typically utilizes either the Boc or Fmoc strategy. The Boc strategy uses a 1% cross-linked polystyrene resin. The standard protecting group for α-amino functions is the tert-butyloxycarbonyl (Boc) group. This group can be removed with dilute solutions of strong acids such as 25% trifluoroacetic acid (TFA). The next Boc-amino acid is typically coupled to the amino acyl resin using dicyclohexylcarbodiimide (DCC). Following completion of the assembly, the peptide-resin is treated with anhydrous HF to cleave the benzyl ester link and liberate the free peptide. Side-chain functional groups are usually blocked during synthesis by benzyl-derived blocking groups, which are also cleaved by HF. The free peptide is then extracted from the resin with a suitable solvent, purified and characterized. Newly synthesized peptides can be purified, for example, by gel filtration, HPLC, partition chromatography and/or ion-exchange chromatography, and may be characterized by, for example, mass spectrometry or amino acid sequence analysis. In the Boc strategy, C-terminal amidated peptides can be obtained using benzhydrylamine or methylbenzhydrylamine resins, which yield peptide amides directly upon cleavage with HF.

In the procedures discussed above, the selectivity of the side-chain blocking groups and of the peptide-resin link depends upon the differences in the rate of acidolytic cleavage. Orthoganol systems have been introduced in which the side-chain blocking groups and the peptide-resin link are completely stable to the reagent used to remove the α-protecting group at each step of the synthesis. The most common of these methods involves the 9-fluorenylmethyloxycarbonyl (Fmoc) approach. Within this method, the side-chain protecting groups and the peptide-resin link are completely stable to the secondary amines used for cleaving the N-α-Fmoc group. The side-chain protection and the peptide-resin link are cleaved by mild acidolysis. The repeated contact with base makes the Merrifield resin unsuitable for Fmoc chemistry, and p-

alkoxybenzyl esters linked to the resin are generally used. Deprotection and cleavage are generally accomplished using TFA.

Those of ordinary skill in the art will recognize that, in solid phase synthesis, deprotection and coupling reactions must go to completion and the side-chain blocking groups must be stable throughout the entire synthesis. In addition, solid phase synthesis is generally most suitable when peptides are to be made on a small scale.

N-acetylation of the N-terminal residue can be accomplished by reacting the final peptide with acetic anhydride before cleavage from the resin. C-amidation may be accomplished using an appropriate resin such as methylbenzhydrylamine resin using the Boc technology.

For longer modulating agents, recombinant methods are preferred for synthesis. Within such methods, all or part of a modulating agent can be synthesized in living cells, using any of a variety of expression vectors known to those of ordinary skill in the art to be appropriate for the particular host cell. Suitable host cells may include bacteria, yeast cells, mammalian cells, insect cells, plant cells, algae and other animal cells (*e.g.*, hybridoma, CHO, myeloma). The DNA sequences expressed in this manner may encode portions of an endogenous occludin or other adhesion molecule. Such sequences may be prepared based on known cDNA or genomic sequences (*see* Blaschuk et al., *J. Mol. Biol. 211*:679-682, 1990), or from sequences isolated by screening an appropriate library with probes designed based on known occludin sequences. Such screens may generally be performed as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989 (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using oligonucleotide primers in methods well known in the art, to isolate nucleic acid molecules encoding all or a portion of an endogenous adhesion molecule. To generate a nucleic acid molecule encoding a desired modulating agent, an endogenous occludin sequence may be modified using well known techniques. For example, portions encoding one or more CAR sequences may be joined, with or without separation by nucleic acid regions encoding linkers, as discussed above. Alternatively, portions of the desired nucleic acid sequences may be synthesized

using well known techniques, and then ligated together to form a sequence encoding the modulating agent.

As noted above, a modulating agent may comprise one or more cyclic peptides. Such cyclic peptides may contain only one CAR sequence, or may additionally contain one or more other adhesion molecule binding sites, which may or may not be CARs. Such additional sequences may be separated by a linker (*i.e.*, one or more peptides not derived from a CAR sequence or other adhesion molecule binding site, as described previously). Within one such embodiment, a modulating agent comprises a cyclic peptide containing two LYHY (SEQ ID NO:1) sequences. Within another embodiment, a cyclic peptide contains one LYHY (SEQ ID NO:1) and one CAR sequence recognized by a different CAM. In certain preferred embodiments, the second CAR sequence is derived from fibronectin (*i.e.*, RGD); a classical cadherin (*i.e.*, HAV); a claudin or a nonclassical cadherin as described above.

Cyclic peptides containing at least one occludin CAR sequence may be covalently linked to either cyclic or linear peptides containing at least one CAR sequence recognized by a different CAM, as described previously. Using a linker, cyclic LYHY-containing peptides and other cyclic or linear peptide or protein sequences may be joined head-to-tail (*i.e.*, the linker may be covalently attached to the carboxyl or amino group of each peptide sequence), head-to-side chain and/or tail-to-side chain. Modulating agents comprising one or more linkers may form linear or branched structures. Within one embodiment, modulating agents having a branched structure comprise multiple different CAR sequences, such as various combinations of LYHY (SEQ ID NO:1), RGD, HAV, claudin CAR sequence(s) and/or nonclassical cadherin CAR sequence(s).

In addition to the CAR sequence(s), cyclic peptides generally comprise at least one additional residue, such that the size of the cyclic peptide ring ranges from 5 to about 15 residues, preferably from 5 to 10 residues. Such additional residue(s) may be present on the N-terminal and/or C-terminal side of a CAR sequence, and may be derived from sequences that flank the endogenous occludin CAR sequence with or without amino acid substitutions and/or other modifications. Alternatively, additional

residues present on one or both sides of the CAR sequence(s) may be unrelated to an endogenous sequence (*e.g.*, residues that facilitate cyclization).

Within certain preferred embodiments, as discussed below, relatively small cyclic peptides that do not contain significant sequences flanking the LYHY sequence are preferred for modulating occludin mediated cell adhesion. Such peptides may contain an N-acetyl group and a C-amide group (*e.g.*, the 6-residue ring N-Ac-CLYHYC-NH_2 (SEQ ID NO:3). Within the context of the present invention, underlined peptide sequences indicate cyclic peptides, wherein the cyclization is performed by any suitable method as provided herein.

Within other preferred embodiments, a cyclic peptide may contain sequences that flank the LYHY (SEQ ID NO:1) sequence in a native occludin molecule on one or both sides. Such sequences may result in increased potency. Suitable flanking sequences include, but are not limited to, the endogenous sequence present in naturally occurring occludin. To facilitate the preparation of cyclic peptides having increased potency, nuclear magnetic resonance (NMR) and computational techniques may be used to determine the conformation of a peptide that confers increased potency, as described above.

Cyclic peptides as described herein may comprise residues of L-amino acids, D-amino acids, or any combination thereof. A cyclic peptide may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (*e.g.*, benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (*e.g.*, acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (*e.g.*, methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Preferred derivatives include amino acids having an N-acetyl group (such that the amino group that represents the N-terminus of the linear peptide prior to cyclization is acetylated) and/or a C-terminal amide group (*i.e.*, the carboxy terminus of the linear peptide prior to cyclization is amidated). Residues other than common amino acids that may be present with a cyclic peptide include, but are not

limited to, penicillamine, β,β-tetramethylene cysteine, β,β-pentamethylene cysteine, β-mercaptopropionic acid, β,β-pentamethylene-β-mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptoproline, ornithine, diaminobutyric acid, α-aminoadipic acid, m-aminomethylbenzoic acid and α,β-diaminopropionic acid.

Cyclic peptides as described herein may be synthesized by methods well known in the art, including recombinant DNA methods and chemical synthesis. Following synthesis of a linear peptide (utilizing methods described herein), with or without N-acetylation and/or C-amidation, cyclization may be achieved by any of a variety of techniques well known in the art. Within one embodiment, a bond may be generated between reactive amino acid side chains. For example, a disulfide bridge may be formed from a linear peptide comprising two thiol-containing residues by oxidizing the peptide using any of a variety of methods. Within one such method, air oxidation of thiols can generate disulfide linkages over a period of several days using either basic or neutral aqueous media. The peptide is used in high dilution to minimize aggregation and intermolecular side reactions. This method suffers from the disadvantage of being slow but has the advantage of only producing H_2O as a side product. Alternatively, strong oxidizing agents such as I_2 and $K_3Fe(CN)_6$ can be used to form disulfide linkages. Those of ordinary skill in the art will recognize that care must be taken not to oxidize the sensitive side chains of Met, Tyr, Trp or His. Cyclic peptides produced by this method require purification using standard techniques, but this oxidation is applicable at acid pHs. By way of example, strong oxidizing agents can be used to perform the cyclization shown below (SEQ ID NOs:19 and 20), in which the underlined portion is cyclized:

FmocCysAsp(t-Bu)GlyTyr(t-Bu)ProLys(Boc)Asp(t-Bu)CysLys(t-Bu)Gly-OMe

→

Fmoc<u>CysAsp(t-Bu)GlyTyr(t-Bu)ProLys(Boc)Asp(t-Bu)Cys</u>Lys(t-Bu)Gly-OMe

Oxidizing agents also allow concurrent deprotection/oxidation of suitable S-protected linear precursors to avoid premature, nonspecific oxidation of free

cysteine, as shown below (SEQ ID Nos:21 and 22), where X and Y = S-Trt or S-Acm:

BocCys(X)GlyAsnLeuSer(t-Bu)Thr(t-Bu)Cys(Y)MetLeuGlyOH →
BocCysGlyAsnLeuSer(t-Bu)Thr(t-Bu)CysMetLeuGlyOH

DMSO, unlike I_2 and $K_3Fe(CN)_6$, is a mild oxidizing agent which does not cause oxidative side reactions of the nucleophilic amino acids mentioned above. DMSO is miscible with H_2O at all concentrations, and oxidations can be performed at acidic to neutral pHs with harmless byproducts. Methyltrichlorosilane-diphenylsulfoxide may alternatively be used as an oxidizing agent, for concurrent deprotection/oxidation of S-Acm, S-Tacm or S-t-Bu of cysteine without affecting other nucleophilic amino acids. There are no polymeric products resulting from intermolecular disulfide bond formation. In the example below (SEQ ID NOs:23 and 24), X is Acm, Tacm or t-Bu:

H-Cys(X)TyrIleGlnAsnCys(X)ProLeuGly-NH_2 →
H-CysTyrIleGlnAsnCysProLeuGly-NH_2

Suitable thiol-containing residues for use in such oxidation methods include, but are not limited to, cysteine, β,β-dimethyl cysteine (penicillamine or Pen), β,β-tetramethylene cysteine (Tmc), β,β-pentamethylene cysteine (Pmc), β-mercaptopropionic acid (Mpr), β,β-pentamethylene-β-mercaptopropionic acid (Pmp), 2-mercaptobenzene, 2-mercaptoaniline and 2-mercaptoproline. Peptides containing such residues are illustrated by the following representative formulas, in which the underlined portion is cyclized, N-acetyl groups are indicated by N-Ac and C-terminal amide groups are represented by -NH_2:

i) N-Ac-Cys-Leu-Tyr-His-Tyr-Cys-NH_2 (SEQ ID NO:25)

ii) H-Cys-Leu-Tyr-His-Tyr-Cys-OH (SEQ ID NO:26)

iii) N-Ac-Cys-Gln-Tyr-Leu-Tyr-His-Tyr-Cys-NH_2 (SEQ ID NO:27)

iv) H-Cys-Gln-Tyr-Leu-Tyr-His-Tyr-Cys-OH (SEQ ID NO:28)

v) N-Ac-Cys-Tyr-Leu-Tyr-His-Tyr-Cys-NH_2 (SEQ ID NO:29)

vi) H-Cys-Tyr-Leu-Tyr-His-Tyr-Cys-OH (SEQ ID NO:30)

vii) N-Ac-Cys-Leu-Tyr-His-Tyr-Pen-NH_2 (SEQ ID NO:31)

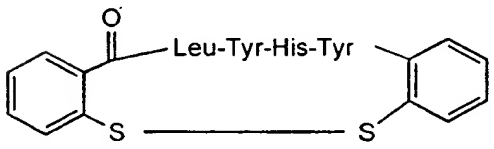
viii) N-Ac-Tmc-Leu-Tyr-His-Tyr-Cys-NH_2 (SEQ ID NO:32)

ix) N-Ac-Pmc-Leu-Tyr-His-Tyr-Cys-NH_2 (SEQ ID NO:33)

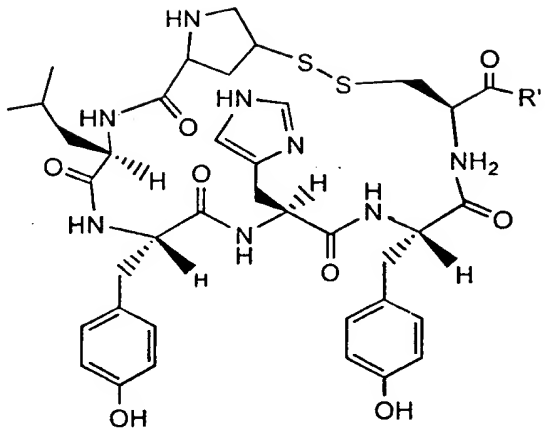
x) N-Ac-Mpr-Leu-Tyr-His-Tyr-Cys-NH_2 (SEQ ID NO:34)

xi) N-Ac-Pmp-Leu-Tyr-His-Tyr-Cys-NH_2 (SEQ ID NO:35)

xii)



xiii)



It will be readily apparent to those of ordinary skill in the art that, within each of these representative formulas, any of the above thiol-containing residues may be employed in place of one or both of the thiol-containing residues recited.

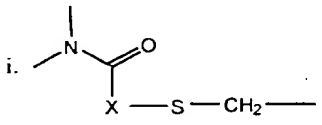
Within another embodiment, cyclization may be achieved by amide bond formation. For example, a peptide bond may be formed between terminal functional groups (*i.e.*, the amino and carboxy termini of a linear peptide prior to cyclization). Two such cyclic peptides are YLYHY (SEQ ID NO:18) and QYLYHY (SEQ ID NO:17). Within another such embodiment, the cyclic peptide comprises a D-amino acid (*e.g.*, yLYHY; SEQ ID NO:18). Alternatively, cyclization may be accomplished by linking one terminus and a residue side chain or using two side chains, as in KLYHYD (SEQ ID NO:36) or KQYLYHYD (SEQ ID NO:37), with or without an N-terminal acetyl group and/or a C-terminal amide. Residues capable of forming a lactam bond include lysine, ornithine (Orn), α-amino adipic acid, m-aminomethylbenzoic acid, α,β-diaminopropionic acid, glutamate or aspartate.

Methods for forming amide bonds are well known in the art and are based on well established principles of chemical reactivity. Within one such method, carbodiimide-mediated lactam formation can be accomplished by reaction of the carboxylic acid with DCC, DIC, EDAC or DCCI, resulting in the formation of an O-acylurea that can be reacted immediately with the free amino group to complete the cyclization. The formation of the inactive N-acylurea, resulting from O→N migration,

can be circumvented by converting the O-acylurea to an active ester by reaction with an N-hydroxy compound such as 1-hydroxybenzotriazole, 1-hydroxysuccinimide, 1-hydroxynorbornene carboxamide or ethyl 2-hydroximino-2-cyanoacetate. In addition to minimizing O→N migration, these additives also serve as catalysts during cyclization and assist in lowering racemization. Alternatively, cyclization can be performed using the azide method, in which a reactive azide intermediate is generated from an alkyl ester via a hydrazide. Hydrazinolysis of the terminal ester necessitates the use of a t-butyl group for the protection of side chain carboxyl functions in the acylating component. This limitation can be overcome by using diphenylphosphoryl acid (DPPA), which furnishes an azide directly upon reaction with a carboxyl group. The slow reactivity of azides and the formation of isocyanates by their disproportionation restrict the usefulness of this method. The mixed anhydride method of lactam formation is widely used because of the facile removal of reaction by-products. The anhydride is formed upon reaction of the carboxylate anion with an alkyl chloroformate or pivaloyl chloride. The attack of the amino component is then guided to the carbonyl carbon of the acylating component by the electron donating effect of the alkoxy group or by the steric bulk of the pivaloyl chloride t-butyl group, which obstructs attack on the wrong carbonyl group. Mixed anhydrides with phosphoric acid derivatives have also been successfully used. Alternatively, cyclization can be accomplished using activated esters. The presence of electron withdrawing substituents on the alkoxy carbon of esters increases their susceptibility to aminolysis. The high reactivity of esters of p-nitrophenol, N-hydroxy compounds and polyhalogenated phenols has made these "active esters" useful in the synthesis of amide bonds. The last few years have witnessed the development of benzotriazolyloxytris-(dimethylamino)phosphonium hexafluorophosphonate (BOP) and its congeners as advantageous coupling reagents. Their performance is generally superior to that of the well established carbodiimide amide bond formation reactions.

Within a further embodiment, a thioether linkage may be formed between the side chain of a thiol-containing residue and an appropriately derivatized α-amino acid. By way of example, a lysine side chain can be coupled to bromoacetic acid

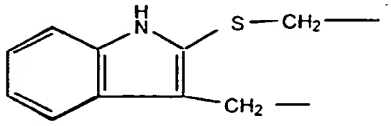
through the carbodiimide coupling method (DCC, EDAC) and then reacted with the side chain of any of the thiol containing residues mentioned above to form a thioether linkage. In order to form dithioethers, any two thiol containing side-chains can be reacted with dibromoethane and diisopropylamine in DMF. Examples of thiol-containing linkages are shown below:

i. 

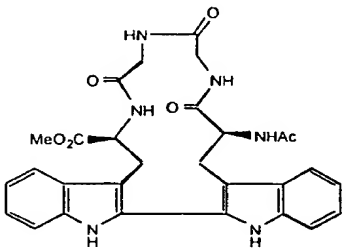
$X = (CH_2)_4$

$= CH_2$

$= -C_6H_4-CH_2$

ii. 

Cyclization may also be achieved using δ_1,δ_1-Ditryptophan (*i.e.*, Ac-Trp-Gly-Gly-Trp-OMe) (SEQ ID NO:38), as shown below:



Representative structures of cyclic peptides are provided in Figure 3. Within Figure 3, certain cyclic peptides having the ability to modulate cell adhesion (shown on the left) are paired with similar inactive structures (on the right). The structures and formulas recited herein are provided solely for the purpose of illustration, and are not intended to limit the scope of the cyclic peptides described herein.

As noted above, instead of (or in addition to) an occludin CAR sequence,

a modulating agent may comprise an antibody, or antigen-binding fragment thereof, that specifically binds to a occludin CAR sequence. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a occludin CAR sequence (with or without flanking amino acids) if it reacts at a detectable level with a peptide containing that sequence, and does not react detectably with peptides containing a different CAR sequence or a sequence in which the order of amino acid residues in the occludin CAR sequence and/or flanking sequence is altered. Such antibody binding properties may be assessed using an ELISA, as described by Newton et al., *Develop. Dynamics 197*:1-13, 1993.

Polyclonal and monoclonal antibodies may be raised against an occludin CAR sequence using conventional techniques. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the occludin CAR sequence is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). The smaller immunogens (*i.e.*, less than about 20 amino acids) should be joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. Following one or more injections, the animals are bled periodically. Polyclonal antibodies specific for the CAR sequence may then be purified from such antisera by, for example, affinity chromatography using the modulating agent or antigenic portion thereof coupled to a suitable solid support.

Monoclonal antibodies specific for the occludin CAR sequence may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity from spleen cells obtained from an animal immunized as described above. The spleen cells are immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. Single colonies are selected and their culture supernatants tested for binding activity against the modulating agent or antigenic portion thereof. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies, with or without the use of various techniques known in the art to enhance the yield. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. Antibodies having the desired activity may generally be identified using immunofluorescence analyses of tissue sections, cell or other samples where the target occludin is localized.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988; *see* especially page 309) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns (Harlow and Lane, 1988, pages 628-29).

EVALUATION OF MODULATING AGENT ACTIVITY

As noted above, modulating agents as described herein are capable of modulating occludin-mediated cell adhesion. The ability of an agent to modulate cell adhesion may generally be evaluated *in vitro* by assaying the effect on endothelial and/or epithelial cell adhesion using, for example, any of a variety of immunostaining protocols and/or plating assays. In general, a modulating agent is an inhibitor of cell adhesion if contact of the test cells with the modulating agent results in a discernible disruption of cell adhesion using one or more representative assays provided herein. Modulating agents that enhance cell adhesion (*e.g.*, agents comprising multiple LYHY (SEQ ID NO:1) sequences and/or linked to a support molecule or material) are considered to be modulators of cell adhesion if they are capable of promoting cell adhesion, as judged by plating assays to assess either endothelial or epithelial cell adhesion to a modulating agent attached to a support material, such as tissue culture plastic.

The ability of an agent to modulate cell adhesion may generally be

evaluated *in vivo* by assessing the effect on vascular permeability utilizing the Miles assay (McClure et al., J. *Pharmacological & Toxicological Methods 32*:49-52, 1994). Briefly, a candidate modulating agent may be dissolved in phosphate buffered saline (PBS) at a concentration of 100 μg/ml. Adult rats may be given 100 μl subdermal injections of each peptide solution into their shaved backs, followed 15 minutes later by a single 250 μl injection of 1% Evans blue dissolved in PBS into their tail veins. The subdermal injection sites may be visually monitored for the appearance of blue dye. Once the dye appears (about 15 minutes after injection), each subdermal injection site may be excised, weighed, and placed in 1 ml dimethylformamide for 24 hours to extract the dye. The optical density of the dye extracts may then be determined at 620 nm. In general, the injection of 0.1 ml of modulating agent (at a concentration of 0.1 mg/ml) into the backs of rats causes an increase of dye accumulation at the injection sites of at least 50%, as compared to dye accumulation at sites into which PBS has been injected.

The effect of a modulating agent on endothelial cell adhesion may generally be evaluated using immunolocalization techniques. Human aortic endothelial cells (HAEC) may be cultured on fibronectin-coated coverslips (fibronectin may be obtained from Sigma, St. Louis, MO) according to the procedures of Jaffe et al., *J. Clin. Invest. 52*:2745-2756, 1973. Briefly, human endothelial cells may be maintained in EGM (endothelial cell growth medium; Clonetics, San Diego, CA) and used for experiments at passage 4. Confluent cultures of HAEC may be exposed to either a candidate modulating agent (final concentration 100 μg/ml EGM), or EGM alone for 1 hour. The cells are then be fixed for 30 minutes at 4°C in 95% ethanol, followed by fixation in acetone for 1 minute at 4°C (Furuse et al., *J. Cell Biol. 123:1777-1788*, 1993). After fixation, the cells may be probed with either mouse anti-VE-cadherin antibodies (Hemeris, Sassenage, France; diluted 1:250 in 0.1% dried skim milk powder dissolved in PBS), or rabbit anti-occludin antibodies (Zymed, South San Francisco, CA; diluted 1:300 in 0.1% dried skim milk powder dissolved in PBS) for 1 hour at 37°C. The cells may then be washed with 0.1% dried skim milk powder dissolved in PBS (three washes, 5 minutes/wash), and probed with secondary antibodies (donkey anti-mouse Cy3, or donkey anti-rabbit Cy5 diluted 1:250 in 0.1% dried skim milk powder

dissolved in PBS; Jackson Immunoresearch Laboratories Inc., Westgrove, PA) for 1 hour at 37°C. The cells may then be washed again with in 0.1% dried skim milk powder dissolved in PBS and mounted in a solution composed of 50% glycerol and 50% PBS to which phenylenediamine (Sigma, St. Louis, MO) has been added to a final concentration of 1 mg/ml. The sample may then be analyzed using a Bio-Rad MRC 1000 confocal microscope with Laser Sharp software version 2.1T (Bio-Rad, Hercules, CA). In general, 0.1 mg/ml of modulating agent results in the appearance of intercellular gaps within the monolayer cultures and a decrease of at least 50% in the surface expression of occludin and VE-cadherin, as compared to monolayer cultures that were not exposed to the modulating agent.

Within certain cell adhesion assays, the addition of a modulating agent to cells that express occludin results in disruption of cell adhesion. An "occludin-expressing cell," as used herein, may be any type of cell that expresses occludin on the cell surface at a detectable level, using standard techniques such as immunocytochemical protocols (*e.g.*, Blaschuk and Farookhi, *Dev. Biol. 136*:564-567, 1989). Occludin-expressing cells include endothelial, epithelial and/or cancer cells. For example, such cells may be plated under standard conditions that, in the absence of modulating agent, permit cell adhesion. In the presence of modulating agent (*e.g.*, 100 µg/mL), disruption of cell adhesion may be determined visually within 24 hours, by observing retraction of the cells from one another.

Within another such assay, the effect of a modulating agent on normal rat kidney (NRK) cells may be evaluated. According to a representative procedure, NRK cells (ATCC #1571-CRL) may be plated at 10 – 20,000 cells per 35mm tissue culture flasks containing DMEM with 10% FCS and sub-cultured periodically (Laird et al., *J. Cell Biol. 131*:1193-1203, 1995). Cells may be harvested and replated in 35mm tissue culture flasks containing 1 mm coverslips and incubated until 50–65% confluent (24-36 hours). At this time, coverslips may be transferred to a 24-well plate, washed once with fresh DMEM and exposed to modulating agent at a concentration of, for example, 0.1 mg/mL for 24 hours. Fresh modulating agent may then be added, and the cells left for an additional 24 hours. Cells may be fixed with 100% methanol for 10

minutes and then washed three times with PBS. Coverslips may be blocked for 1 hour in 2% BSA/PBS and incubated for a further 1 hour in the presence of rabbit anti-occludin antibody ((Zymed, South San Francisco, CA) and mouse anti-E-cadherin antibody (Transduction Labs. 1:250 dilution). Primary and secondary antibodies may be diluted in 2% BSA/PBS. Following incubation in the primary antibody, coverslips may be washed three times for 5 minutes each in PBS and incubated for 1 hour with donkey anti-mouse Cy3 and donkey anti-rabbit Cy5 (Jackson Immunoresearch Laboratories Inc., Westgrove, PA) for 1 hour at 37°C. Following further washes in PBS (3 x 5 min) coverslips can be mounted and viewed by confocal microscopy.

In the absence of modulating agent, NRK cells form characteristic tightly adherent monolayers with a cobblestone morphology in which cells display a polygonal shape. NRK cells that are treated with a modulating agent that disrupts occludin-mediated cell adhesion may assume a non-polygonal and elongated morphology (*i.e.*, a fibroblast-like shape) within 48 hours of treatment with 0.1 mg/mL of modulating agent. Gaps appear in confluent cultures of such cells. In addition, 0.1 mg/mL of such a modulating agent reproducibly induces a readily apparent reduction in cell surface staining of occludin and E-cadherin, as judged by immunofluorescence microscopy (Laird et al., *J. Cell Biol. 131*:1193-1203, 1995), of at least 75% within 48 hours.

A third cell adhesion assay involves evaluating the effect of a modulating agent on permeability of adherent endothelial cell monolayers. The effects of a modulating agent on the permeability of endothelial cell monolayers may be assessed utilizing the protocols of Ehringer et al., *J. Cell. Physiol. 167*:562-569, 1996. HAEC can be seeded onto inserts in 24-well plates (Becton-Dickenson, Franklin Lake, NJ) and cultured in EGM. Confluent cell monolayers may be exposed to either modulating agent (final concentration 100 µg/ml EGM), or EGM alone for 1 hour. The inserts may then be transferred to 24-chamber plates (Becton-Dickenson) for permeability assays. Perfusate (0.5% bovine serum albumin, fraction V (Sigma) dissolved in 15 mM HEPES, pH 7.4) and FITC-Dextran (50 µg/ml HEPES buffer; MW 12 kDa; Sigma) may be added to each well (1 ml/well and 50 µl/well, respectively), and the cells incubated at 37°C for 30 min. Aliquots of 100 µl may then be removed

from the lower chamber and the optical density of the solution determined at a wavelength of 450 nm. In general, the presence of 100 µg/mL modulating agent that enhances the permeability of endothelial cell monolayers results in a statistically significant increase in the amount of marker in the receptor compartment after 1 hour.

Yet another assay evaluates the effect of an occludin modulating agent on the electrical resistance across a monolayer of cells. For example, Madin Darby canine kidney (MDCK) cells can be exposed to the modulating agent dissolved in medium (*e.g.*, at a final concentration of 0.5 mg/ml for a period of 24 hours). The effect on electrical resistance can be measured using standard techniques. This assay evaluates the effect of a modulating agent on tight junction formation in epithelial cells. In general, the presence of 500 µg/mL modulating agent should result in a statistically significant increase or decrease in electrical resistance after 24 hours.

MODULATING AGENT MODIFICATION AND FORMULATIONS

A modulating agent as described herein may, but need not, be linked to one or more additional molecules. In particular, as discussed below, it may be beneficial for certain applications to link multiple modulating agents (which may, but need not, be identical) to a support material, such as a single molecule (*e.g.*, keyhole limpet hemocyanin) or a solid support, such as a polymeric matrix (which may be formulated as a membrane or microstructure, such as an ultra thin film), a container surface (*e.g.*, the surface of a tissue culture plate or the interior surface of a bioreactor), or a bead or other particle, which may be prepared from a variety of materials including glass, plastic or ceramics. For certain applications, biodegradable support materials are preferred, such as cellulose and derivatives thereof, collagen, spider silk or any of a variety of polyesters (*e.g.*, those derived from hydroxy acids and/or lactones) or sutures (*see* U.S. Patent No. 5,245,012). Within certain embodiments, modulating agents and molecules comprising other CAR sequence(s) (*e.g.*, an HAV sequence) may be attached to a support such as a polymeric matrix, preferably in an alternating pattern.

Suitable methods for linking a modulating agent to a support material will depend upon the composition of the support and the intended use, and will be

readily apparent to those of ordinary skill in the art. Attachment may generally be achieved through noncovalent association, such as adsorption or affinity or, preferably, via covalent attachment (which may be a direct linkage between a modulating agent and functional groups on the support, or may be a linkage by way of a cross-linking agent). Attachment of a modulating agent by adsorption may be achieved by contact, in a suitable buffer, with a solid support for a suitable amount of time. The contact time varies with temperature, but is generally between about 5 seconds and 1 day, and typically between about 10 seconds and 1 hour.

Covalent attachment of a modulating agent to a molecule or solid support may generally be achieved by first reacting the support material with a bifunctional reagent that will also react with a functional group, such as a hydroxyl, thiol, carboxyl, ketone or amino group, on the modulating agent. For example, a modulating agent may be bound to an appropriate polymeric support or coating using benzoquinone, by condensation of an aldehyde group on the support with an amine and an active hydrogen on the modulating agent or by condensation of an amino group on the support with a carboxylic acid on the modulating agent. A preferred method of generating a linkage is via amino groups using glutaraldehyde. A modulating agent may be linked to cellulose via ester linkages. Similarly, amide linkages may be suitable for linkage to other molecules such as keyhole limpet hemocyanin or other support materials. Multiple modulating agents and/or molecules comprising other CAR sequences may be attached, for example, by random coupling, in which equimolar amounts of such molecules are mixed with a matrix support and allowed to couple at random.

Although modulating agents as described herein may preferentially bind to specific tissues or cells, and thus may be sufficient to target a desired site *in vivo*, it may be beneficial for certain applications to include an additional targeting agent. Accordingly, a targeting agent may also, or alternatively, be linked to a modulating agent to facilitate targeting to one or more specific tissues. As used herein, a "targeting agent," may be any substance (such as a compound or cell) that, when linked to a modulating agent enhances the transport of the modulating agent to a target tissue,

thereby increasing the local concentration of the modulating agent. Targeting agents include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include serum hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and those drugs and proteins that bind to a desired target site. Among the many monoclonal antibodies that may serve as targeting agents are anti-TAC, or other interleukin-2 receptor antibodies; 9.2.27 and NR-ML-05, reactive with the 250 kilodalton human melanoma-associated proteoglycan; and NR-LU-10, reactive with a pancarcinoma glycoprotein. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')2, -Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers.

For certain embodiments, it may be beneficial to also, or alternatively, link a drug to a modulating agent. As used herein, the term "drug" refers to any bioactive agent intended for administration to a mammal to prevent or treat a disease or other undesirable condition. Drugs include hormones, growth factors, proteins, peptides and other compounds. The use of certain specific drugs within the context of the present invention is discussed below.

Modulating agents as described herein may be present within a pharmaceutical composition. A pharmaceutical composition comprises one or more modulating agents in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide) and/or preservatives. Within yet other embodiments, compositions of the present invention may be

formulated as a lyophilizate. One or more modulating agents (alone or in combination with a targeting agent and/or drug) may, but need not, be encapsulated within liposomes using well known technology. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration.

For certain embodiments, as discussed below, a pharmaceutical composition may further comprise a modulator of cell adhesion that is mediated by one or more molecules other than occludin. Such modulators may generally be prepared as described above, incorporating one or more non-occludin CAR sequences and/or antibodies thereto in place of the occludin CAR sequence and antibodies. Such compositions are particularly useful for situations in which it is desirable to inhibit cell adhesion mediated by multiple cell adhesion molecules, such as cadherins (*e.g.*, classical cadherins, E-cadherin, Dsg and Dsc); integrins; members of the immunoglobulin supergene family (such as N-CAM and PECAM); and claudins. Preferred CAR sequences for use within such a modulator include HAV, RGD, and CAR sequences of claudins, VE-cadherin, dsg and dsc.

A pharmaceutical composition may also, or alternatively, contain one or more drugs, which may be linked to a modulating agent or may be free within the composition. Virtually any drug may be administered in combination with a modulating agent as described herein, for a variety of purposes as described below. Examples of types of drugs that may be administered with a modulating agent include analgesics, anesthetics, antianginals, antifungals, antibiotics, anticancer drugs (*e.g.*, taxol or mitomycin C), antiinflammatories (*e.g.*, ibuprofen and indomethacin), anthelmintics, antidepressants, antidotes, antiemetics, antihistamines, antihypertensives, antimalarials, antimicrotubule agents (*e.g.*, colchicine or vinca alkaloids), antimigraine agents, antimicrobials, antiphsychotics, antipyretics, antiseptics, anti-signaling agents (*e.g.*, protein kinase C inhibitors or inhibitors of intracellular calcium mobilization), antiarthritics, antithrombin agents, antituberculotics, antitussives, antivirals, appetite suppressants, cardioactive drugs, chemical dependency drugs, cathartics,

chemotherapeutic agents, coronary, cerebral or peripheral vasodilators, contraceptive agents, depressants, diuretics, expectorants, growth factors, hormonal agents, hypnotics, immunosuppression agents, narcotic antagonists, parasympathomimetics, sedatives, stimulants, sympathomimetics, toxins (*e.g.*, cholera toxin), tranquilizers and urinary antiinfectives.

For imaging purposes, any of a variety of diagnostic agents may be incorporated into a pharmaceutical composition, either linked to a modulating agent or free within the composition. Diagnostic agents include any substance administered to illuminate a physiological function within a patient, while leaving other physiological functions generally unaffected. Diagnostic agents include metals, radioactive isotopes and radioopaque agents (*e.g.*, gallium, technetium, indium, strontium, iodine, barium, bromine and phosphorus-containing compounds), radiolucent agents, contrast agents, dyes (*e.g.*, fluorescent dyes and chromophores) and enzymes that catalyze a colorimetric or fluorometric reaction. In general, such agents may be attached using a variety of techniques as described above, and may be present in any orientation.

The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of modulating agent following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a modulating agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane (*see, e.g.*, European Patent Application 710,491 A). Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of modulating agent release. The amount of modulating agent contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented).

Appropriate dosages and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the modulating agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Within particularly preferred embodiments of the invention, a modulating agent or pharmaceutical composition as described herein may be administered at a dosage ranging from 0.001 to 50 mg/kg body weight, preferably from 0.1 to 20 mg/kg, on a regimen of single or multiple daily doses. For topical administration, a cream typically comprises an amount of modulating agent ranging from 0.00001% to 1%, preferably from 0.0001% to 0.2% and more preferably from 0.01% to 0.1%. Fluid compositions typically contain an amount of modulating agent ranging from 10 ng/ml to 5 mg/ml, preferably from 10 μg to 2 mg/mL. Appropriate dosages may generally be determined using experimental models and/or clinical trials. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

MODULATING AGENT METHODS OF USE

In general, the modulating agents and compositions described herein may be used for modulating the adhesion of occludin-expressing cells *in vitro* and/or *in vivo*. As noted above, modulating agents for purposes that involve the disruption of occludin-mediated cell adhesion may comprise an occludin CAR sequence, multiple occludin CAR sequences in close proximity and/or an antibody (or an antigen-binding fragment thereof) that recognizes the occludin CAR sequence. When it is desirable to also disrupt cell adhesion mediated by other adhesion molecules, a modulating agent may additionally comprise one or more CAR sequences bound by such adhesion molecules (and/or antibodies or fragments thereof that bind such sequences), preferably separated from each other and from the occludin CAR sequence by linkers. As noted above, such linkers may or may not comprise one or more amino acids. For enhancing

cell adhesion, a modulating agent may contain multiple occludin CAR sequences or antibodies (or fragments), preferably separated by linkers, and/or may be linked to a single molecule or to a support material as described above.

Certain methods involving the disruption of cell adhesion as described herein have an advantage over prior techniques in that they permit the passage of molecules that are large and/or charged across barriers of occludin-expressing cells. As described in greater detail below, modulating agents as described herein may also be used to disrupt or enhance cell adhesion in a variety of other contexts. Within each of the methods described herein, one or more modulating agents may generally be administered alone, or within a pharmaceutical composition. In each specific method described herein, as noted above, a targeting agent may be employed to increase the local concentration of modulating agent at the target site.

The present invention also provides methods for increasing vasopermeability in a mammal by administering one or more modulating agents or pharmaceutical compositions. It has been found, within the context of the present invention, that endothelial cell adhesion can be disrupted by linear and cyclic peptides containing the occludin CAR sequence, LYHY (SEQ ID NO:1). Within blood vessels, endothelial cell adhesion results in decreased vascular permeability. Accordingly, modulating agents that disrupt occludin-mediated endothelial cell adhesion as described herein, can increase vascular permeability and thus may facilitate drug delivery to previously inaccessible tissues, such as the brain.

Certain preferred modulating agents for use within such methods are H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof having one or more C-terminal, N-terminal and/or side chain modifications. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). In one particularly preferred embodiment, a modulating agent is capable of disrupting cell adhesion mediated by multiple adhesion molecules. For example, a single branched modulating agent (or multiple agents linked to a single molecule or support material) may disrupt occludin and cadherin mediated

cell adhesion, thereby disrupting tight junctions and adherens junctions. Multi-functional modulating agents comprising the occludin CAR sequence LYHY (SEQ ID NO:1) joined (preferable by a linker) to one or more of a classical cadherin CAR sequence, a claudin CAR sequence, an OB-cadherin CAR sequence and/or a VE-cadherin CAR sequence are also preferred. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Preferred antibody modulating agents that may be used in conjunction with the occludin modulating agents include Fab fragments directed against an N-cadherin CAR sequence, such as FHLRAHAVDINGNQV-NH_2 (SEQ ID NO:4).

Within certain embodiments, preferred modulating agents for use within such methods include peptides capable of decreasing both endothelial and tumor cell adhesion. Such modulating agents may be used to facilitate the penetration of anti-tumor therapeutic or diagnostic agents (*e.g.*, monoclonal antibodies) through endothelial cell permeability barriers and tumor barriers. In one particularly preferred embodiment, a modulating agent is capable of disrupting cell adhesion mediated by multiple adhesion molecules. For example, a single branched modulating agent (or multiple agents linked to a single molecule or support material) may disrupt occludin, claudin, VE-cadherin, Dsc and Dsg mediated cell adhesion. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Preferred antibody modulating agents that may be used in conjunction with the occludin modulating agents include Fab fragments directed against either an N-cadherin CAR sequence, such as FHLRAHAVDINGNQV-NH_2 (SEQ ID NO:4), or an E-cadherin CAR sequence, such as LFSHAVSSNG-NH_2 (SEQ ID NO:39).

Treatment with a modulating agent may be appropriate, for example, prior to administration of an anti-tumor therapeutic or diagnostic agent (*e.g.*, a monoclonal antibody or other macromolecule), an antimicrobial agent or an anti-inflammatory agent, in order to increase the concentration of such agents in the vicinity of the target tumor, organism or inflammation without increasing the overall dose to the

patient. Modulating agents for use within such methods may be linked to a targeting agent to further increase the local concentration of modulating agent, although systemic administration of a vasoactive agent even in the absence of a targeting agent increases the perfusion of certain tumors relative to other tissues. Suitable targeting agents include antibodies and other molecules that specifically bind to tumor cells or to components of structurally abnormal blood vessels. For example, a targeting agent may be an antibody that binds to a fibrin degradation product or a cell enzyme such as a peroxidase that is released by granulocytes or other cells in necrotic or inflamed tissues.

Administration via intravenous injection or transdermal administration is generally preferred. Effective dosages are generally sufficient to increase localization of a subsequently administered diagnostic or therapeutic agent to an extent that improves the clinical efficacy of therapy of accuracy of diagnosis to a statistically significant degree. Comparison may be made between treated and untreated tumor host animals to whom equivalent doses of the diagnostic or therapeutic agent are administered. In general, dosages range as described above.

Within further aspects, the present invention provides methods in which cell adhesion is diminished. In one such aspect, methods for reducing unwanted cellular adhesion by administering a modulating agent are provided. Unwanted cellular adhesion can occur between tumor cells, between tumor cells and normal cells or between normal cells as a result of surgery, injury, chemotherapy, disease, inflammation or other condition jeopardizing cell viability or function. Preferred modulating agents for use within such methods include H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). In addition, a modulating agent may comprise one or more of a claudin CAR sequence, a CAR sequence for a nonclassical cadherin (such as VE-cadherin, OB-cadherin, dsc or dsg), RGD sequence, and/or HAV sequence separated from an occludin CAR sequence via a linker. Alternatively, separate modulators of cadherin- and integrin-mediated cell adhesion may be administered in

conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Topical administration of the modulating agent(s) is generally preferred, but other means may also be employed. Preferably, a fluid composition for topical administration (comprising, for example, physiological saline) comprises an amount of modulating agent as described above, and more preferably from 10μg/mL to 1mg/mL. Creams may generally be formulated as described above. Topical administration in the surgical field may be given once at the end of surgery by irrigation of the wound or as an intermittent or continuous irrigation with the use of surgical drains in the post-operative period or by the use of drains specifically inserted in an area of inflammation, injury or disease in cases where surgery does not need to be performed. Alternatively, parenteral or transcutaneous administration may be used to achieve similar results.

Within another such aspect, methods are provided for enhancing the delivery of a drug through the skin of a mammal. Transdermal delivery of drugs is a convenient and non-invasive method that can be used to maintain relatively constant blood levels of a drug. In general, to facilitate drug delivery via the skin, it is necessary to perturb adhesion between the epithelial cells (keratinocytes) and the endothelial cells of the microvasculature. Using currently available techniques, only small, uncharged molecules may be delivered across skin *in vivo*. The methods described herein are not subject to the same degree of limitation. Accordingly, a wide variety of drugs may be transported across the epithelial and endothelial cell layers of skin, for systemic or topical administration. Such drugs may be delivered to melanomas or may enter the blood stream of the mammal for delivery to other sites within the body.

To enhance the delivery of a drug through the skin, a modulating agent as described herein and a drug are contacted with the skin surface. Preferred modulating agents for use within such methods include H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). Multifunctional modulating agents comprising an

occludin CAR sequence linked to one or more of a claudin CAR sequence, VE-cadherin CAR sequence, dsc CAR sequence, dsg CAR sequence, RGD sequence, and/or HAV sequence may also be used to disrupt cell adhesion. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Contact may be achieved by direct application of the modulating agent, generally within a composition formulated as a cream or gel, or using any of a variety of skin contact devices for transdermal application (such as those described in European Patent Application No. 566,816 A; U.S. Patent No. 5,613,958; U.S. Patent No. 5,505,956). A skin patch provides a convenient method of administration (particularly for slow-release formulations). Such patches may contain a reservoir of modulating agent and drug separated from the skin by a membrane through which the drug diffuses. Within other patch designs, the modulating agent and drug may be dissolved or suspended in a polymer or adhesive matrix that is then placed in direct contact with the patient's skin. The modulating agent and drug may then diffuse from the matrix into the skin. Modulating agent(s) and drug(s) may be contained within the same composition or skin patch, or may be separately administered, although administration at the same time and site is preferred. In general, the amount of modulating agent administered via the skin varies with the nature of the condition to be treated or prevented, but may vary as described above. Such levels may be achieved by appropriate adjustments to the device used, or by applying a cream formulated as described above. Transfer of the drug across the skin and to the target tissue may be predicted based on *in vitro* studies using, for example, a Franz cell apparatus, and evaluated *in vivo* by appropriate means that will be apparent to those of ordinary skill in the art. As an example, monitoring of the serum level of the administered drug over time provides an easy measure of the drug transfer across the skin.

Transdermal drug delivery as described herein is particularly useful in situations in which a constant rate of drug delivery is desired, to avoid fluctuating blood levels of a drug. For example, morphine is an analgesic commonly used immediately following surgery. When given intermittently in a parenteral form (intramuscular,

intravenous), the patient usually feels sleepy during the first hour, is well during the next 2 hours and is in pain during the last hour because the blood level goes up quickly after the injection and goes down below the desirable level before the 4 hour interval prescribed for re-injection is reached. Transdermal administration as described herein permits the maintenance of constant levels for long periods of time (*e.g.*, days), which allows adequate pain control and mental alertness at the same time. Insulin provides another such example. Many diabetic patients need to maintain a constant baseline level of insulin which is different from their needs at the time of meals. The baseline level may be maintained using transdermal administration of insulin, as described herein. Antibiotics may also be administered at a constant rate, maintaining adequate bactericidal blood levels, while avoiding the high levels that are often responsible for the toxicity (*e.g.*, levels of gentamycin that are too high typically result in renal toxicity).

Drug delivery by the methods of the present invention also provide a more convenient method of drug administration. For example, it is often particularly difficult to administer parenteral drugs to newborns and infants because of the difficulty associated with finding veins of acceptable caliber to catheterize. However, newborns and infants often have a relatively large skin surface as compared to adults. Transdermal drug delivery permits easier management of such patients and allows certain types of care that can presently be given only in hospitals to be given at home. Other patients who typically have similar difficulties with venous catheterization are patients undergoing chemotherapy or patients on dialysis. In addition, for patients undergoing prolonged therapy, transdermal administration as described herein is more convenient than parenteral administration.

Transdermal administration as described herein also allows the gastrointestinal tract to be bypassed in situations where parenteral uses would not be practical. For example, there is a growing need for methods suitable for administration of therapeutic small peptides and proteins, which are typically digested within the gastrointestinal tract. The methods described herein permit administration of such compounds and allow easy administration over long periods of time. Patients who have

problems with absorption through their gastrointestinal tract because of prolonged ileus or specific gastrointestinal diseases limiting drug absorption may also benefit from drugs formulated for transdermal application as described herein.

Further, there are many clinical situations where it is difficult to maintain compliance. For example, patients with mental problems (*e.g.*, patients with Alzheimer's disease or psychosis) are easier to manage if a constant delivery rate of drug is provided without having to rely on their ability to take their medication at specific times of the day. Also patients who simply forget to take their drugs as prescribed are less likely to do so if they merely have to put on a skin patch periodically (*e.g.*, every 3 days). Patients with diseases that are without symptoms, like patients with hypertension, are especially at risk of forgetting to take their medication as prescribed.

For patients taking multiple drugs, devices for transdermal application such as skin patches may be formulated with combinations of drugs that are frequently used together. For example, many heart failure patients are given digoxin in combination with furosemide. The combination of both drugs into a single skin patch facilitates administration, reduces the risk of errors (taking the correct pills at the appropriate time is often confusing to older people), reduces the psychological strain of taking "so many pills," reduces skipped dosage because of irregular activities and improves compliance.

The methods described herein are particularly applicable to humans, but also have a variety of veterinary uses, such as the administration of growth factors or hormones (*e.g.*, for fertility control) to an animal.

As noted above, a wide variety of drugs may be administered according to the methods provided herein. Some examples of drug categories that may be administered transdermally include anti-inflammatory drugs (*e.g.*, in arthritis and in other condition) such as all NSAID, indomethacin, prednisone, etc.: analgesics (especially when oral absorption is not possible, such as after surgery, and when parenteral administration is not convenient or desirable), including morphine, codeine, Demerol, acetaminophen and combinations of these (*e.g.*, codeine plus

acetaminophen); antibiotics such as Vancomycin (which is not absorbed by the GI tract and is frequently given intravenously) or a combination of INH and Rifampicin (*e.g.*, for tuberculosis); anticoagulants such as heparin (which is not well absorbed by the GI tract and is generally given parenterally, resulting in fluctuation in the blood levels with an increased risk of bleeding at high levels and risks of inefficacy at lower levels) and Warfarin (which is absorbed by the GI tract but cannot be administered immediately after abdominal surgery because of the normal ileus following the procedure); antidepressants (*e.g.*, in situations where compliance is an issue as in Alzheimer's disease or when maintaining stable blood levels results in a significant reduction of anti-cholinergic side effects and better tolerance by patients), such as amitriptylin, imipramin, prozac, etc.; antihypertensive drugs (*e.g.*, to improve compliance and reduce side effects associated with fluctuating blood levels), such as diuretics and beta-blockers (which can be administered by the same patch; *e.g.*, furosemide and propanolol); antipsychotics (*e.g.*, to facilitate compliance and make it easier for care giver and family members to make sure that the drug is received), such as haloperidol and chlorpromazine; and anxiolytics or sedatives (*e.g.*, to avoid the reduction of alertness related to high blood levels after oral administration and allow a continual benefit throughout the day by maintaining therapeutic levels constant).

Numerous other drugs may be administered as described herein, including naturally occurring and synthetic hormones, growth factors, proteins and peptides. For example, insulin and human growth hormone, growth factors like erythropoietin, interleukins and inteferons may be delivered via the skin.

Kits for administering a drug via the skin of a mammal are also provided within the present invention. Such kits generally comprise a device for transdermal application (*e.g.*, a skin patch) in combination with, or impregnated with, one or more modulating agents. A drug may additionally be included within such kits.

Within a related aspect, the use of modulating agents as described herein to increase skin permeability may also facilitate sampling of the blood compartment by passive diffusion, permitting detection and/or measurement of the levels of specific molecules circulating in the blood. For example, application of one or more modulating

agents to the skin, via a skin patch as described herein, permits the patch to function like a sponge to accumulate a small quantity of fluid containing a representative sample of the serum. The patch is then removed after a specified amount of time and analyzed by suitable techniques for the compound of interest (*e.g.*, a medication, hormone, growth factor, metabolite or marker). Alternatively, a patch may be impregnated with reagents to permit a color change if a specific substance (*e.g.*, an enzyme) is detected. Substances that can be detected in this manner include, but are not limited to, illegal drugs such as cocaine, HIV enzymes, glucose and PSA. This technology is of particular benefit for home testing kits.

Within a further aspect, methods are provided for enhancing delivery of a drug to a tumor in a mammal, comprising administering a modulating agent in combination with a drug to a tumor-bearing mammal. Preferred modulating agents for use within such methods include H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). In one particularly preferred embodiment, a modulating agent is capable of disrupting cell adhesion mediated by multiple adhesion molecules. For example, a single branched modulating agent (or multiple agents linked to a single molecule or support material) may disrupt occludin, classical cadherin, integrin, and nonclassical cadherin (*e.g.*, Dsc and Dsg) mediated cell adhesion, thereby disrupting tight junctions, adherens junctions, and desmosomes. Multifunctional modulating agents comprising an occludin CAR sequence linked to an RGD sequence and/or CAR sequence(s) for one or more of a classical cadherin, claudin or nonclassical cadherin (*e.g.*, OB-cadherin, VE-cadherin, dsc or dsg) may be used to disrupt cell adhesion. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Preferred antibody modulating agents that may be used in conjunction with the occludin modulating agents include Fab fragments directed against either an N-cadherin CAR sequence (such as FHLRAHAVDINGNQV-

NH_2; SEQ ID NO:4) or an E-cadherin CAR sequence LFSHAVSSNG-NH_2 (SEQ ID NO:39).

Preferably, the modulating agent and the drug are formulated within the same composition or drug delivery device prior to administration. In general, a modulating agent may enhance drug delivery to any tumor, and the method of administration may be chosen based on the type of target tumor. For example, injection or topical administration as described above may be preferred for melanomas and other accessible tumors (*e.g.*, metastases from primary ovarian tumors may be treated by flushing the peritoneal cavity with the composition). Other tumors (*e.g.*, bladder tumors) may be treated by injection of the modulating agent and the drug (such as mitomycin C) into the site of the tumor. In other instances, the composition may be administered systemically, and targeted to the tumor using any of a variety of specific targeting agents. Suitable drugs may be identified by those of ordinary skill in the art based upon the type of cancer to be treated (*e.g.*, mitomycin C for bladder cancer). In general, the amount of modulating agent administered varies with the method of administration and the nature of the tumor, within the typical ranges provided above, preferably ranging from about 1μg/mL to about 2 mg/mL, and more preferably from about 10μg/mL to 1mg/mL. Transfer of the drug to the target tumor may be evaluated by appropriate means that will be apparent to those of ordinary skill in the art. Drugs may also be labeled (*e.g.*, using radionuclides) to permit direct observation of transfer to the target tumor using standard imaging techniques.

Within a related aspect, the present invention provides methods for treating cancer and/or inhibiting metastasis in a mammal. Cancer tumors are solid masses of cells, growing out of control, which require nourishment via blood vessels. The formation of new capillaries is a prerequisite for tumor growth and the emergence of metastases. Administration of modulating agents as described herein may disrupt the growth of such blood vessels, thereby providing effective therapy for the cancer and/or inhibiting metastasis. Modulating agents may also be used to treat leukemias. Preferred modulating agents for use within such methods include H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents

comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). In one particularly preferred embodiment, a modulating agent is capable of disrupting cell adhesion mediated by multiple adhesion molecules. For example, a single branched modulating agent (or multiple agents linked to a single molecule or support material) may disrupt occludin, classical cadherin, integrin, Dsc and Dsg mediated cell adhesion, thereby disrupting tight junctions, adherens junctions, focal contacts and desmosomes. Multifunctional modulating agents comprising the occludin CAR sequence linked to one or more of a claudin CAR sequence, VE-cadherin CAR sequence, dsc CAR sequence, dsg CAR sequence, RGD sequence, OB-cadherin CAR sequence and/or HAV sequence may be used to disrupt cell adhesion. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Preferred antibody modulating agents that may be used in conjunction with the occludin modulating agents include Fab fragments directed against either an N-cadherin CAR sequence, such as FHLRAHAVDINGNQV-NH_2 (SEQ ID NO:4), or an E-cadherin CAR sequence, such as LFSHAVSSNG-NH_2 (SEQ ID NO:39). A modulating agent may be administered alone (*e.g.*, via the skin) or within a pharmaceutical composition. For melanomas and certain other accessible tumors, injection or topical administration as described above may be preferred. For ovarian cancers, flushing the peritoneal cavity with a composition comprising one or more modulating agents may prevent metastasis of ovarian tumor cells. Other tumors (*e.g.*, bladder tumors, bronchial tumors or tracheal tumors) may be treated by injection of the modulating agent into the cavity. In other instances, the composition may be administered systemically, and targeted to the tumor using any of a variety of specific targeting agents, as described above. In general, the amount of modulating agent administered varies depending upon the method of administration and the nature of the cancer, but may vary within the ranges identified above. The effectiveness of the cancer treatment or inhibition of metastasis may be evaluated using well known clinical observations, such as monitoring the level of serum

tumor markers (*e.g.*, CEA or PSA).

Within a further related aspect, a modulating agent may be used to inhibit angiogenesis (*i.e.*, the growth of blood vessels from pre-existing blood vessels) in a mammal. Inhibition of angiogenesis may be beneficial, for example, in patients afflicted with diseases such as cancer or arthritis. Preferred modulating agents for use within such methods include H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). In one particularly preferred embodiment, a modulating agent is capable of disrupting cell adhesion mediated by multiple adhesion molecules. For example, a single branched modulating agent (or multiple agents linked to a single molecule or support material) may disrupt occludin, classical cadherin, and integrin mediated cell adhesion, thereby disrupting tight junctions, adherens junctions, and focal contacts. Multifunctional modulating agents comprising the occludin CAR sequence linked to one or more of a claudin CAR sequence, VE-cadherin CAR sequence, dsc CAR sequence, dsg CAR sequence, RGD sequence, and/or HAV sequence may be used to disrupt cell adhesion. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Preferred antibody modulating agents that may be used in conjunction with the occludin modulating agents include Fab fragments directed against an N-cadherin CAR sequence, such as FHLRAHAVDINGNQV-NH_2 (SEQ ID NO:4).

The effect of a particular modulating agent on angiogenesis may generally be determined by evaluating the effect of the agent on blood vessel formation. Such a determination may generally be performed, for example, using a chick chorioallantoic membrane assay (Iruela-Arispe et al., *Molecular Biology of the Cell* 6:327-343, 1995). Briefly, a modulating agent may be embedded in a mesh composed of vitrogen at one or more concentrations (*e.g.*, ranging from about 5 to 50 μg/mesh). The mesh(es) may then be applied to chick chorioallantoic membranes. After 24 hours,

the effect of the modulating agent may be determined using computer assisted morphometric analysis. A modulating agent should inhibit angiogenesis by at least 25% at a concentration of 50 μg/mesh.

The addition of a targeting agent as described above may be beneficial, particularly when the administration is systemic. Suitable modes of administration and dosages depend upon the condition to be prevented or treated but, in general, administration by injection is appropriate. Dosages may vary as described above. The effectiveness of the inhibition may be evaluated grossly by assessing the inability of the tumors to maintain their growth and microscopically by observing an absence of nerves at the periphery of the tumor.

In yet another related aspect, the present invention provides methods for inducing apoptosis in an occludin-expressing cell. In general, patients afflicted with cancer may benefit from such treatment. Preferred modulating agents for use within such methods include H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). In one particularly preferred embodiment, a modulating agent is capable of disrupting cell adhesion mediated by multiple adhesion molecules. For example, a single branched modulating agent (or multiple agents linked to a single molecule or support material) may disrupt occludin, classical cadherin, and integrin mediated cell adhesion, thereby disrupting tight junctions, adherens junctions, and focal contacts. Multifunctional modulating agents comprising the occludin CAR sequence linked to one or more of a claudin CAR sequence, nonclassical cadherin CAR sequence (*e.g.*, VE-cadherin, OB-cadherin, dsc or dsg), RGD sequence, and/or HAV sequence may be used to disrupt cell adhesion. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Preferred antibody modulating agents that may be used in conjunction with the occludin modulating agents include Fab fragments directed against either an N-cadherin CAR sequence, such as

FHLRAHAVDINGNQV-NH_2 (SEQ ID NO:4), or an E-cadherin CAR sequence, such as LFSHAVSSNG-NH_2 (SEQ ID NO:39).

Administration of modulating agents to induce apoptosis may be topical, via injection or by other means, and the addition of a targeting agent may be beneficial, particularly when the administration is systemic. Suitable modes of administration and dosages depend upon the location and nature of the cells for which induction of apoptosis is desired but, in general, dosages may vary as described above. A biopsy may be performed to evaluate the level of induction of apoptosis.

The present invention also provides methods for enhancing drug delivery to the central nervous system of a mammal. The blood/brain barrier is largely impermeable to most neuroactive agents, and delivery of drugs to the brain of a mammal often requires invasive procedures. Using a modulating agent as described herein, however, delivery may be by, for example, systemic administration of a modulating agent-drug-targeting agent combination, injection of a modulating agent (alone or in combination with a drug and/or targeting agent) into the carotid artery or application of a skin patch comprising a modulating agent to the head of the patient. Certain preferred modulating agents for use within such methods are H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). In one particularly preferred embodiment, a modulating agent is capable of disrupting cell adhesion mediated by multiple adhesion molecules. For example, a single branched modulating agent (or multiple agents linked to a single molecule or support material) may disrupt occludin and cadherin mediated cell adhesion, thereby disrupting tight junctions and adherens junctions. Multifunctional modulating agents comprising the occludin CAR sequence linked to one or more of a claudin CAR sequence, VE-cadherin CAR sequence, OB-cadherin CAR sequence and/or HAV sequence may be used to disrupt cell adhesion. Alternatively, a separate modulator of non-occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either

within the same pharmaceutical composition or separately. Preferred antibody modulating agents that may be used in conjunction with the occludin modulating agents include Fab fragments directed against the N-cadherin CAR sequence FHLRAHAVDINGNQV-NH_2 (SEQ ID NO:4).

In general, the amount of modulating agent administered varies as described above, and with the method of administration and the nature of the condition to be treated or prevented. Transfer of the drug to the central nervous system may be evaluated by appropriate means that will be apparent to those of ordinary skill in the art, such as magnetic resonance imaging (MRI) or PET scan (positron emitted tomography).

Within further aspects, modulating agents as described herein may be used for modulating the immune system of a mammal in any of several ways. Modulating agents may generally be used to modulate specific steps within cellular interactions during an immune response or during the dissemination of malignant lymphocytes. For example, a modulating agent as described herein may be used to treat diseases associated with excessive generation of otherwise normal T cells. Without wishing to be bound by any particular theory, it is believed that the interaction of occludin on maturing T cells and B cell subsets contributes to protection of these cells from programmed cell death. A modulating agent may decrease such interactions, leading to the induction of programmed cell death. Accordingly, modulating agents may be used to treat certain types of diabetes and rheumatoid arthritis, particularly in young children where the cadherin expression on thymic pre-T cells is greatest.

Modulating agents may also be administered to patients afflicted with certain skin disorders (such as cutaneous lymphomas), acute B cell leukemia and excessive immune reactions involving the humoral immune system and generation of immunoglobulins, such as allergic responses and antibody-mediated graft rejection. In addition, patients with circulating cadherin-positive malignant cells (*e.g.*, during regimes where chemotherapy or radiation therapy is eliminating a major portion of the malignant cells in bone marrow and other lymphoid tissue) may benefit from treatment with a modulating agent. Such treatment may also benefit patients undergoing transplantation with peripheral blood stem cells.

Certain preferred modulating agents for use within such methods include those that comprise one or more additional CAR sequences, such as a claudin CAR sequence, HAV, RGD, a VE-cadherin CAR sequence and/or KYSFNYDGSE (SEQ ID NO:63). As noted above, such additional sequence(s) may be separated from an occludin CAR sequence via a linker. Alternatively, a separate modulator of cadherin-, claudin-, integrin- and/or N-CAM-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately.

Within the above methods, the modulating agent(s) are preferably administered systemically (usually by injection) or topically. A modulating agent may be linked to a targeting agent. For example, targeting to the bone marrow may be beneficial. A suitable dosage is sufficient to effect a statistically significant reduction in the population of B and/or T cells that express cadherin and/or an improvement in the clinical manifestation of the disease being treated. Typical dosages generally range as described above.

In certain other aspects, the present invention provides methods for enhancing adhesion of occludin-expressing cells. Within certain embodiments, a modulating agent may be linked to a solid support, resulting in a matrix that comprises multiple modulating agents. Within one such embodiment, the support is a polymeric matrix to which modulating agents and molecules comprising other CAR sequence(s) are attached (*e.g.*, modulating agents and molecules comprising HAV and RGD sequences may be attached to the same matrix, preferably in an alternating pattern). Such matrices may be used in contexts in which it is desirable to enhance adhesion mediated by multiple cell adhesion molecules. Alternatively, the modulating agent itself may comprise multiple occludin CAR sequences or antibodies (or fragments thereof), separated by linkers as described above. Either way, the modulating agent(s) function as a "biological glue" to bind multiple occludin-expressing cells within a variety of contexts.

Within one such aspect, modulating agents comprising multiple occludin CAR sequences and/or multiple modulating agents linked to a single molecule or

support material may be used to enhance wound healing and/or reduce scar tissue in a mammal. Peptides that may be linked to a support, and/or to one another via a linker, to generate a suitable modulating agent include, but are not limited to, H-QYLYHYCVVD-OH (SEQ ID NO:2) and H-CLYHYC-OH (SEQ ID NO:3) and modulating agents comprising such sequences or derivatives thereof. Preferred antibody modulating agents include Fab fragments directed against either H-QYLYHYCVVD-OH (SEQ ID NO:2) or H-CLYHYC-OH (SEQ ID NO:3). Modulating agents that are linked to a biocompatible and biodegradable matrix such as cellulose or collagen are particularly preferred. For use within such methods, a modulating agent should have a free amino or hydroxyl group. The modulating agents are generally administered topically to the wound, where they may facilitate closure of the wound and may augment, or even replace, stitches. Similarly, administration of matrix-linked modulating agents may facilitate cell adhesion in skin grafting and prosthetic implants, and may prolong the duration and usefulness of collagen injection. In general, the amount of matrix-linked modulating agent administered to a wound, graft or implant site varies with the severity of the wound and/or the nature of the wound, graft, or implant, but may vary as discussed above. Multifunctional modulating agents comprising the occludin CAR sequence linked to one or more of a claudin CAR sequence, nonclassical cadherin CAR sequence (*e.g.*, VE-cadherin, OB-cadherin, dsc or dsg), RGD sequence, and/or HAV sequence may be used as potent stimulators of wound healing and/or to reduce scar tissue. Alternatively, one or more separate modulators of cadherin-, claudin-, integrin-, Dsc- and/or Dsg-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately.

Within another aspect, one or more modulating agents may be linked to the interior surface of a tissue culture plate or other cell culture support, such as for use in a bioreactor. Such linkage may be performed by any suitable technique, as described above. Modulating agents linked in this fashion may generally be used to immobilize occludin-expressing cells. For example, dishes or plates coated with one or more modulating agents may be used to immobilize occludin-expressing cells within a variety

of assays and screens. Within bioreactors (*i.e.*, systems for large scale production of cells or organoids), modulating agents may generally be used to improve cell attachment and stabilize cell growth. Modulating agents may also be used within bioreactors to support the formation and function of highly differentiated organoids derived, for example, from dispersed populations of fetal mammalian cells. Bioreactors containing biomatrices of modulating agent(s) may also be used to facilitate the production of specific proteins.

Modulating agents as described herein may be used within a variety of bioreactor configurations. In general, a bioreactor is designed with an interior surface area sufficient to support large numbers of adherent cells. This surface area can be provided using membranes, tubes, microtiter wells, columns, hollow fibers, roller bottles, plates, dishes, beads or a combination thereof. A bioreactor may be compartmentalized. The support material within a bioreactor may be any suitable material known in the art; preferably, the support material does not dissolve or swell in water. Preferred support materials include, but are not limited to, synthetic polymers such as acrylics, vinyls, polyethylene, polypropylene, polytetrafluoroethylene, nylons, polyurethanes, polyamides, polysulfones and poly(ethylene terephthalate); ceramics; glass and silica.

Within certain aspects, modulating agents may be used to stimulate the formation of epithelial cell tight junctions. It has been found, within the context of the present invention, that certain peptide modulating agents that inhibit adhesion of endothelial cells, may stimulate adhesion of epithelial cells. Such agents include H-QYLYHYCVVD-COOH and N-Ac-CLYHYC-NH_2, and other such agents may be readily identified using the assays provided herein. Agents that stimulate adhesion of epithelial cells may be used in any context in which such selective stimulation is desirable. For example, diarrhea is known to be the result of toxins that break down tight junctions in intestinal epithelial cells (*see, e.g.*, Philpott et al., *Infect. Immun. 66*:1680-1687, 1998; Spitz et al., *Am. J. Physiol. 268*:G374-379, 1995; Fasano et al., *Proc. Natl. Acad. Sci. USA, 88*:5242-5246, 1991). Modulating agents that stimulate tight junction formation may be administered to patients as described herein to inhibit

diarrhea. Such agents may, for example, be administered orally.

Other aspects of the present invention provide methods that employ antibodies raised against the modulating agents for diagnostic and assay purposes. Assays typically involve using an antibody to detect the presence or absence of occludin (free or on the surface of a cell), or proteolytic fragment containing the EC2 domain in a suitable biological sample, such as tumor or normal tissue biopsies, blood, lymph node, serum or urine samples, or other tissue, homogenate, or extract thereof obtained from a patient.

There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect a target molecule in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, the assay may be performed in a Western blot format, wherein a protein preparation from the biological sample is submitted to gel electrophoresis, transferred to a suitable membrane and allowed to react with the antibody. The presence of the antibody on the membrane may then be detected using a suitable detection reagent, as described below.

In another embodiment, the assay involves the use of antibody immobilized on a solid support to bind to the target occludin, or a proteolytic fragment containing the EC2 domain and encompassing the CAR sequence, and remove it from the remainder of the sample. The bound occludin may then be detected using a second antibody or reagent that contains a reporter group. Alternatively, a competitive assay may be utilized, in which the occludin is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled occludin to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result, indicative of the level of the occludin in the sample.

The solid support may be any material known to those of ordinary skill in the art to which the antibody may be attached, such as a test well in a microtiter plate, a nitrocellulose filter or another suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic such as polystyrene or

polyvinylchloride. The antibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature.

In certain embodiments, the assay for detection of occludin in a sample is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that the occludin within the sample is allowed to bind to the immobilized antibody (a 30 minute incubation time at room temperature is generally sufficient). Unbound sample is then removed from the immobilized occludin-antibody complexes and a second antibody (containing a reporter group such as an enzyme, dye, radionuclide, luminescent group, fluorescent group or biotin) capable of binding to a different site on the occludin is added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products. Standards and standard additions may be used to determine the level of occludin in a sample, using well known techniques.

The present invention also provides kits for use in such immunoassays. Such kits generally comprise one or more antibodies, as described above. In addition, one or more additional compartments or containers of a kit generally enclose elements, such as reagents, buffers and/or wash solutions, to be used in the immunoassay.

Within further aspects, modulating agents or antibodies (or fragments thereof) may be used to facilitate cell identification and sorting *in vitro* or imaging *in vivo*, permitting the selection of cells expressing occludin (or different occludin levels).

Preferably, the modulating agent(s) or antibodies for use in such methods are linked to a detectable marker. Suitable markers are well known in the art and include radionuclides, luminescent groups, fluorescent groups, enzymes, dyes, constant immunoglobulin domains and biotin. Within one preferred embodiment, a modulating agent linked to a fluorescent marker, such as fluorescein, is contacted with the cells, which are then analyzed by fluorescence activated cell sorting (FACS).

Antibodies or fragments thereof may also be used within screens of combinatorial or other nonpeptide-based libraries to identify other compounds capable of modulating occludin-mediated cell adhesion. Such screens may generally be performed using an ELISA or other method well known to those of ordinary skill in the art that detect compounds with a shape and structure similar to that of the modulating agent. In general, such screens may involve contacting an expression library producing test compounds with an antibody, and detecting the level of antibody bound to the candidate compounds. Compounds for which the antibody has a higher affinity may be further characterized as described herein, to evaluate the ability to modulate occludin-mediated cell adhesion.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

Preparation of Representative Cyclic Peptides

This Example illustrates the solid phase synthesis of representative linear and cyclic peptides as modulating agents.

The peptides were assembled on methylbenzhydrylamine resin (MBHA resin) for the C-terminal amide peptides. The traditional Merrifield resins were used for any C-terminal acid peptides. Bags of a polypropylene mesh material were filled with the resin and soaked in dichloromethane. The resin packets were washed three times with 5% diisopropylethylamine in dichloromethane and then washed with dichloromethane. The packets are then sorted and placed into a Nalgene bottle containing a solution of the amino acid of interest in dichloromethane. An equal amount of diisopropylcarbodiimide (DIC) in dichloromethane was added to activate the coupling reaction. The bottle was shaken for one hour to ensure completion of the reaction. The reaction mixture was discarded and the packets washed with DMF. The N-α-Boc was removed by acidolysis using a 55% TFA in dichloromethane for 30 minutes leaving the TFA salt of the α-amino group. The bags were washed and the synthesis completed by repeating the same procedure while substituting for the corresponding amino acid at the coupling step. Acetylation of the N-terminal was performed by reacting the peptide resins with a solution of acetic anhydride in dichloromethane in the presence of diisopropylethylamine. The peptide was then side-chain deprotected and cleaved from the resin at 0°C with liquid HF in the presence of anisole as a carbocation scavenger.

The crude peptides were purified by reversed-phase high-performance liquid chromatography. Purified linear precursors of the cyclic peptides were solubilized in 75% acetic acid at a concentration of 2-10mg/mL. A 10% solution of iodine in methanol was added dropwise until a persistent coloration was obtained. A 5% ascorbic acid solution in water was then added to the mixture until discoloration. The disulfide bridge containing compounds were then purified by HPLC and characterized by analytical HPLC and by mass spectral analysis.

EXAMPLE 2

Establishment of a Model System for Assessing Endothelial Cell Adhesion

This Example illustrates an endothelial cell adhesion assay for evaluating the effects of occludin-modulating agents on endothelial cell adhesion.

A. Cell Culture

Human aortic endothelial cells (HAEC) were cultured on fibronectin (Sigma, St. Louis, MO) according to the procedures of Jaffe et al., *J. Clin. Invest. 52*:2745-2756, 1973. Cells were maintained in EGM (endothelial cell growth medium; Clonetics, San Diego, CA) and used for experiments at passage 4.

B. Occludin and VE-cadherin Immunolocalization Methods

HAEC were cultured on fibronectin-coated coverslips. Confluent cultures of HAEC were exposed to linear peptides (final concentration 100 μg/ml EGM), or EGM alone for 1 hour. The cells were then fixed for 30 minutes at 4°C in 95% ethanol, followed by fixation in acetone for 1 minute at 4°C (Furuse et al., *J. Cell Biol. 123:1777-1788*, 1993). After fixation, the cells were allowed to air dry at room temperature. The cells were probed with either mouse anti-VE-cadherin antibodies (Hemeris, Sassenage, France; diluted 1:250 in 0.1% dried skim milk powder dissolved in PBS), or rabbit anti-occludin antibodies (Zymed, South San Francisco, CA; diluted 1:300 in 0.1% dried skim milk powder dissolved in PBS) for 1 hour at 37°C. The cells were then washed with 0.1% dried skim milk powder dissolved in PBS (three washes, 5 minutes/wash), and probed with secondary antibodies (donkey anti-mouse Cy3, or donkey anti-rabbit Cy5 diluted 1:250 in 0.1% dried skim milk powder dissolved in PBS; Jackson Immunoresearch Laboratories Inc., Westgrove, PA) for 1 hour at 37°C. The cells were washed again with in 0.1% dried skim milk powder dissolved in PBS and mounted in a solution composed of 50% glycerol and 50% PBS to which phenylenediamine (Sigma, St. Louis, MO) had been added to a final concentration of 1 mg/ml. The sample were analyzed using a Bio-Rad MRC 1000 confocal microscope

with Laser Sharp software version 2.1T (Bio-Rad, Hercules, CA). Staining for occludin was assigned the pseudo-color red, whereas VE-cadherin staining was assigned pseudo-color green using Confocal Assistant 4.02 software. Immunofluorescence photographs of monolayer cultures of human aortic endothelial cells immunolabeled for occludin (red color) and VE-cadherin (green color) are shown in Figures 4A and 4B. Colocalization of occludin and VE-cadherin is indicated by the yellow color. Arrows indicate gaps between the cells. Note that the endothelial cells retract from one another when cultured in the presence of H-QYLYHYCVVD-OH (SEQ ID NO:2; Figure 4B), indicating that adhesion is decreased between the cells. Furthermore, the cells do not form cobblestone-like monolayers when exposed to this peptide. Also note that surface expression of both VE-cadherin and occludin is greatly reduced in the cells treated with H-QYLYHYCVVD-OH (SEQ ID NO:2), as compared to the VE-cadherin and occludin levels expressed by untreated cells.

EXAMPLE 3

Effect of Representative Modulating Agents on Vasopermeability

This Example illustrates a vasopermeability assay for evaluating the effects of occludin-modulating agents on endothelial cell permeability *in vivo*.

A. Miles Assay for Vascular Permeability

The ability of cyclic and linear peptides to increase vascular permeability was assessed utilizing the Miles assay (McClure et al., *J. Pharmacological & Toxicological Meth. 32*:49-521994). The peptides were dissolved in phosphate buffered saline (PBS) at a concentration of 100 µg/ml. Adult rats were given 100 µl subdermal injections of each peptide solution into their shaved backs, followed 15 minutes later by a single 250 µl injection of 1% Evans blue dissolved in PBS into their tail veins. The subdermal injection sites were visually monitored for the appearance of blue dye. Once the dye appeared (15 minutes after injection), each subdermal injection site was excised, weighed, and placed in 1 ml dimethylformamide for 24 hours to extract the dye. The optical density of the dye extracts was determined at 620 nm. The effects of injecting

either phosphate buffered saline, phosphate buffered saline containing acetyl-QYLYHYCVVD-NH_2 (SEQ ID NO:2) H-QYLYHYCVVD-NH_2 (SEQ ID NO:2), or H-QYLYHYCVVD-OH (SEQ ID NO:2) into sites along the shaved back of a rat on the accumulation of Evans blue at the injection sites is shown in Figure 5. Note that more blue dye has accumulated at the sites where the peptide H-QYLYHYCVVD-OH (SEQ ID NO:2) was injected, as opposed to the sites where either phosphate buffered saline, phosphate buffered saline containing acetyl-QYLYHYCVVD-NH_2 (SEQ ID NO:2), or H-QYLYHYCVVD-NH_2 (SEQ ID NO:2) were injected.

Figure 6 shows a histogram depicting the optical densities of dimethylformamide extracts prepared from the excised injection sites shown in Figure 5. Note that more dye was extracted from the sites injected with H-QYLYHYCVVD-OH (SEQ ID NO:2), than from sites injected with either phosphate buffered saline, acetyl-QYLYHYCVVD-NH_2 (SEQ ID NO:2), or H-QYLYHYCVVD-NH_2 (SEQ ID NO:2).

The effects of injecting either phosphate buffered saline, phosphate buffered saline containing acetyl-CLYHYC-NH_2 (SEQ ID NO:3) or H-CLYHYC-OH (SEQ ID NO:3) into sites along the shaved back of a rat on the accumulation of Evans blue at the injection sites is shown in Figure 7. Figure 8 shows a histogram depicting the optical densities of dimethylformamide extracts prepared from the excised sites of the shaved back of a rat that received injections of either phosphate buffered saline, phosphate buffered saline containing acetyl-CLYHYC-NH_2 (SEQ ID NO:3), or H-CLYHYC-OH (SEQ ID NO:3) at a concentration of 100 µg/ml, followed 15 minutes later by a single injection of Evans blue into the tail vein. Note that more dye was extracted from the sites injected with H-CLYHYC-OH (SEQ ID NO:3), than from sites injected with either phosphate buffered saline, or acetyl-CLYHYC-NH_2 (SEQ ID NO:3).

EXAMPLE 4

Effect of Representative Modulating Agents on

Electrical Resistance Across Cell Monolayer

This Example illustrates an electrical resistance assay for evaluating the effects of occludin-modulating agents on epithelial cell adhesion.

Madin Darby canine kidney (MDCK) cells were plated in Millicells (Millipore, Bedford, MA), at a density of 300,000 cells per Millicell, and cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma, St. Louis, MO) containing 5% fetal calf serum (Sigma, St. Louis, MO). Monolayers were exposed to the modulating agent dissolved in medium at a final concentration of 0.5 mg/ml for a period of 24 hours. The electrical resistance was measured using the EVOM device (World Precision Instruments, Sarasota, FL). At the time of measurement, fresh medium, with or without the modulating agent, may be added to the Millicells.

Figure 9 is a histogram depicting the mean electrical resistance across MDCK cell monolayers cultured for 24 hours in medium alone (Control), or medium containing H-QYLYHYCVVD-NH_2 (SEQ ID NO:2; Peptide 2), H-QYLYHYCVVD-COOH (SEQ ID NO:2; Peptide 3) or N-Ac-CLYHYC-NH_2 (SEQ ID NO:3; Peptide 4) at a concentration of 0.5 mg/ml. Duplicate measurements were taken, and error bars represent the standard deviation. Peptide 2 was found to reduce the electrical resistance, while peptides 3 and 4 increased the electrical resistance across the monolayer, relative to the control. These results demonstrate the ability of occludin modulating agents to modulate the formation of tight junctions in epithelial cells. In particular, certain agents (such as peptides 3 and 4, above) stimulate the formation of tight junctions in epithelial cells.

From the foregoing, it will be evident that although specific embodiments of the invention have been described herein for the purpose of illustrating the invention, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

What is claimed is:

1. A cyclic peptide comprising the sequence LYHY (SEQ ID NO:1), wherein said cyclic peptide modulates occludin-mediated cell adhesion.

2. A cyclic peptide according to claim 1, having the formula:

$$(Z_1)\text{-}(Y_1)\text{-}(X_1)\text{-Leu-Tyr-His-Tyr-}(X_2)\text{-}(Y_2)\text{-}(Z_2)$$

wherein X_1, and X_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds, and wherein X_1 and X_2 independently range in size from 0 to 10 residues, such that the sum of residues contained within X_1 and X_2 ranges from 1 to 12;

wherein Y_1 and Y_2 are independently selected from the group consisting of amino acid residues, and wherein a covalent bond is formed between residues Y_1 and Y_2; and

wherein Z_1 and Z_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds.

3. A cyclic peptide according to claim 2, wherein Z_1 is not present and Y_1 comprises an N-acetyl group.

4. A cyclic peptide according to claim 2, wherein Z_2 is not present and Y_2 comprises a C-terminal amide group.

5. A cyclic peptide according to claim 2, wherein Y_1 and Y_2 are

covalently linked via a disulfide bond.

6. A cyclic peptide according to claim 5, wherein Y_1 and Y_2 are each independently selected from the group consisting of penicillamine, β,β-tetramethylene cysteine, β,β-pentamethylene cysteine, β-mercaptopropionic acid, β,β-pentamethylene-β-mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptoproline and derivatives thereof.

7. A cyclic peptide according to claim 5, wherein Y_1 and Y_2 are cysteine residues or derivatives thereof.

8. A cyclic peptide according to claim 7, further comprising an N-acetyl group.

9. A cyclic peptide according to claim 7, further comprising a C-terminal amide group.

10. A cyclic peptide according to claim 2, wherein Y_1 and Y_2 are covalently linked via an amide bond.

11. A cyclic peptide according to claim 10, wherein said amide bond is formed between terminal functional groups.

12. A cyclic peptide according to claim 10, wherein said amide bond is formed between residue side-chains.

13. A cyclic peptide according to claim 10, wherein said amide bond is formed between one terminal functional group and one residue side chain.

14. A cyclic peptide according to claim 10, wherein:

(a) Y_1 is selected from the group consisting of lysine, ornithine, and derivatives thereof and Y_2 is selected from the group consisting of aspartate, glutamate and derivatives thereof; or

(b) Y_2 is selected from the group consisting of lysine, ornithine and derivatives thereof and Y_1 is selected from the group consisting of aspartate, glutamate and derivatives thereof.

15. A cyclic peptide according to claim 2, wherein Y_1 and Y_2 are covalently linked via a thioether bond.

16. A cyclic peptide according to claim 2, wherein Y_1 and Y_2 are each tryptophan or a derivative thereof, such that said covalent bond generates a $\delta_1\delta_1$-ditryptophan, or a derivative thereof.

17. A cyclic peptide according to claim 2, wherein said cyclic peptide comprises a sequence selected from the group consisting of CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

18. A cell adhesion modulating agent comprising a cyclic peptide according to any one of claims 1-17.

19. A cell adhesion modulating agent according to claim 18 linked to a targeting agent.

20. A cell adhesion modulating agent according to claim 18 linked to a drug.

21. A cell adhesion modulating agent according to claim 18 linked to a solid support.

22. A cell adhesion modulating agent according to claim 21, wherein said solid support is a polymeric matrix.

23. A cell adhesion modulating agent according to claim 21, wherein said solid support is selected from the group consisting of plastic dishes, plastic tubes, sutures, membranes, ultra thin films, bioreactors and microparticles.

24. A cell adhesion modulating agent according to claim 18, further comprising one or more of:

(a) a cell adhesion recognition sequence that is bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

25. A cell adhesion modulating agent according to claim 24, wherein said adhesion molecule is selected from the group consisting of integrins, cadherins, N-CAM, desmogleins, desmocollins, fibronectin, laminin and other extracellular matrix proteins.

26. A cell adhesion modulating agent according to claim 18 linked to a detectable marker.

27. A pharmaceutical composition comprising a cell adhesion modulating agent according to claim 18, in combination with a pharmaceutically acceptable carrier.

28. A composition according to claim 27, further comprising a drug.

29. A composition according to claim 27, wherein said cell adhesion modulating agent is present within a sustained-release formulation.

30. A pharmaceutical composition according to claim 27, further comprising one or more of:

(a) a peptide comprising a cell adhesion recognition sequence that is bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

31. A pharmaceutical composition according to claim 30, wherein said adhesion molecule is selected from the group consisting of integrins, cadherins, N-CAM, desmogleins, desmocollins, fibronectin, laminin and other extracellular matrix proteins.

32. A cell adhesion modulating agent comprising the sequence LYHY (SEQ ID NO:1) or a derivative thereof having one or more C-terminal, N-terminal and/or side chain modifications, wherein the agent comprises no more than 30 consecutive amino acid residues present within a native occludin molecule.

33. A cell adhesion modulating agent according to claim 32, wherein the agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17) and YLYHY (SEQ ID NO:18).

34. A cell adhesion modulating agent comprising an antibody or antigen-binding fragment thereof that specifically binds to an occludin CAR sequence comprising the sequence LYHY (SEQ ID NO:1).

35. A cell adhesion modulating agent according to claim 32 or 34 linked to a targeting agent.

36. A cell adhesion modulating agent according to claim 32 or 34 linked to a drug.

37. A cell adhesion modulating agent according to claim 32 or 34 linked to a solid support.

38. A cell adhesion modulating agent according to claim 37, wherein said solid support is a polymeric matrix.

39. A cell adhesion modulating agent according to claim 37, wherein said solid support is selected from the group consisting of plastic dishes, plastic tubes, sutures, membranes, ultra thin films, bioreactors and microparticles.

40. A cell adhesion modulating agent according to claim 32 or 34, further comprising one or more of:

(a) a cell adhesion recognition sequence that is bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

41. A cell adhesion modulating agent according to claim 40, wherein

said adhesion molecule is selected from the group consisting of integrins, cadherins, N-CAM, desmogleins, desmocollins, fibronectin, laminin and other extracellular matrix proteins.

42. A cell adhesion modulating agent according to claim 32 or 34 linked to a detectable marker.

43. A pharmaceutical composition comprising a cell adhesion modulating agent according to claim 32 or 34, in combination with a pharmaceutically acceptable carrier.

44. A composition according to claim 43, further comprising a drug.

45. A composition according to claim 43, wherein said cell adhesion modulating agent is present within a sustained-release formulation.

46. A pharmaceutical composition according to claim 43, further comprising one or more of:

(a) a peptide comprising a cell adhesion recognition sequence that is bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

47. A pharmaceutical composition according to claim 46, wherein said adhesion molecule is selected from the group consisting of integrins, cadherins, N-CAM, desmogleins, desmocollins, fibronectin, laminin and other extracellular matrix proteins.

48. A method for increasing vasopermeability in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said

modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated endothelial cell adhesion.

49. A method according to claim 48, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45), and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

50. A method for increasing vasopermeability in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent inhibits occludin-mediated cell adhesion.

51. A method according to claim 48 or claim 50, wherein said modulating agent is linked to a targeting agent.

52. A method according to claim 48 or claim 50 wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than occludin.

53. A method according to claim 52, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

54. A method according to claim 52, wherein said antibody or fragment thereof specifically binds to a cadherin cell adhesion recognition sequence comprising a sequence selected from the group consisting of HAV, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

55. A method according to claim 48 or claim 50, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

56. A method according to claim 55, wherein said pharmaceutical composition further comprises a modulator of cell adhesion, comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

57. A method according to claim 56, wherein said cell adhesion recognition sequence comprises the sequence HAV or a claudin CAR sequence.

58. A method according to claim 56, wherein said antibody or fragment thereof specifically binds to a cadherin cell adhesion recognition sequence comprising the sequence HAV or a claudin CAR sequence.

59. A method for reducing unwanted cellular adhesion in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated cell adhesion.

60. A method according to claim 59, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

61. A method for reducing unwanted cellular adhesion in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent inhibits occludin-mediated cell adhesion.

62. A method according to claim 59 or claim 61, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than occludin.

63. A method according to claim 62, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY and EAQ.

64. A method according to claim 62, wherein said cell adhesion recognition sequence is a claudin CAR sequence selected from the group consisting of IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

65. A method according to claim 59 or claim 61, wherein said modulating agent is linked to a targeting agent.

66. A method according to claim 59 or claim 61, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

67. A method according to claim 66, wherein said pharmaceutical composition further comprises a modulator of cell adhesion, comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

68. A method according to claim 67, wherein said cell adhesion recognition sequence comprises the sequence a sequence selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY and EAQ.

69. A method according to claim 67, wherein said cell adhesion

recognition sequence is a claudin CAR sequence selected from the group consisting of IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52.

70. A method for enhancing the delivery of a drug through the skin of a mammal, comprising contacting epithelial cells of a mammal with a cell adhesion modulating agent and a drug, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), wherein said modulating agent inhibits occludin-mediated cell adhesion, and wherein the step of contacting is performed under conditions and for a time sufficient to allow passage of said drug across said epithelial cells.

71. A method for enhancing the delivery of a drug through the skin of a mammal, comprising contacting epithelial cells of a mammal with a cell adhesion modulating agent and a drug, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, wherein said modulating agent inhibits occludin-mediated cell adhesion and wherein the step of contacting is performed under conditions and for a time sufficient to allow passage of said drug across said epithelial cells.

72. A method according to claim 70 or claim 71, wherein said modulating agent passes into the blood stream of said mammal.

73. A method according to claim 70, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal

and/or side chain modifications.

74. A method according to claim 70 or claim 71, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than occludin.

75. A method according to claim 74, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

76. A method according to claim 70 or claim 71, wherein said modulating agent is linked to a targeting agent.

77. A method according to claim 70 or claim 71, wherein said modulating agent is linked to said drug.

78. A method according to claim 70 or claim 71, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

79. A method according to claim 78, wherein said pharmaceutical composition further comprises a modulator of cell adhesion comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion

molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

80. A method according to claim 79, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

81. A method according to claim 79, wherein said antibody or fragment thereof specifically binds to a cell adhesion recognition sequence comprising one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

82. A method according to claim 70 or claim 71, wherein the step of contacting is performed via a skin patch comprising said modulating agent.

83. A method for enhancing the delivery of a drug to a tumor in a mammal, comprising administering to a mammal a cell adhesion modulating agent and a drug, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated cell adhesion.

84. A method for enhancing the delivery of a drug to a tumor in a mammal, comprising administering to a mammal a cell adhesion modulating agent and a drug, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent inhibits occludin-mediated cell adhesion.

85. A method according to claim 83 or claim 84, wherein the tumor is selected from the group consisting of bladder tumors, ovarian tumors and melanomas.

86. A method according to claim 83 or claim 84, wherein said composition is administered to said tumor.

87. A method according to claim 83 or claim 84, wherein said composition is administered systemically.

88. A method according to claim 83, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

89. A method according to claim 83 or claim 84, wherein said modulating agent is linked to a targeting agent.

90. A method according to claim 83 or claim 84, wherein said modulating agent is linked to said drug.

91. A method according to claim 83 or claim 84, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is

separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

92. A method according to claim 91, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

93. A method according to claim 91, wherein said antibody or antigen-binding fragment thereof binds to a cell adhesion recognition sequence comprising a sequence selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

94. A method according to claim 83 or claim 84, wherein said modulating agent and said drug are present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

95. A method according to claim 94, wherein said pharmaceutical composition further comprises a modulator of cell adhesion comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

96. A method according to claim 95, wherein said cell adhesion

recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

97. A method according to claim 95, wherein said antibody or antigen-binding fragment thereof binds to a cell adhesion recognition sequence comprising a sequence selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

98. A method for treating cancer in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated cell adhesion.

99. A method for treating cancer in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent inhibits occludin-mediated cell adhesion.

100. A method according to claim 98 or claim 99, wherein said cancer is selected from the group consisting of carcinomas, leukemia and melanomas.

101. A method according to claim 98, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC

(SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

102. A method according to claim 98 or claim 99, wherein said modulating agent is linked to a targeting agent.

103. A method according to claim 98 or claim 99, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

104. A method according to claim 103, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

105. A method according to claim 98 or claim 99, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

106. A method according to claim 98 or claim 99, wherein said pharmaceutical composition further comprises a modulator of cell adhesion comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion

molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

107. A method according to claim 106, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

108. A method for inhibiting angiogenesis in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated cell adhesion.

109. A method according to claim 108, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

110. A method for inhibiting angiogenesis in a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent inhibits occludin-mediated cell adhesion.

111. A method according to claim 108 or claim 110, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

112. A method according to claim 111, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV, RGD, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

113. A method according to claim 108 or claim 110, wherein said modulating agent is linked to a target agent.

114. A method according to claim 108 or claim 110, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

115. A method according to claim 114, wherein said pharmaceutical composition further comprises a modulator of cell adhesion comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

116. A method according to claim 115, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV, RGD, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

117. A method for enhancing drug delivery to the central nervous system of a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated cell adhesion.

118. A method according to claim 117, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

119. A method for enhancing drug delivery to the central nervous system of a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent inhibits occludin-mediated cell adhesion.

120. A method according to claim 117 or claim 119 wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is

separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

121. A method according to claim 120, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

122. A method according to claim 117 or claim 119, wherein said modulating agent is linked to a targeting agent.

123. A method according to claim 117 or claim 119, wherein said modulating agent is linked to a drug.

124. A method according to claim 117 or claim 119, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

125. A method according to claim 124, wherein said pharmaceutical composition further comprises a modulator of cell adhesion, comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

126. A method according to claim 125, wherein said cell adhesion recognition sequence comprises a sequence selected from the group consisting of HAV,

IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

127. A method for enhancing wound healing in a mammal, comprising contacting a wound in a mammal with a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent enhances occludin-mediated cell adhesion.

128. A method according to claim 127, wherein said modulating agent comprises at least two LYHY (SEQ ID NO:1) sequences separated by a linker.

129. A method according to claim 127, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

130. A method for enhancing wound healing in a mammal, comprising contacting a wound in a mammal with a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent enhances occludin-mediated cell adhesion.

131. A method according to claim 127 or 130, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion

molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

132. A method according to claim 131, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

133. A method according to claim 127 or claim 130, wherein said modulating agent is linked to a targeting agent.

134. A method according to claim 127 or claim 130, wherein said modulating agent is linked to a support material.

135. A method according to claim 127 or claim 130, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

136. A method according to claim 135, wherein said pharmaceutical composition further comprises a modulator of cell adhesion, comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

137. A method according to claim 136, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

138. A method for enhancing adhesion of foreign tissue implanted within a mammal, comprising contacting a site of implantation of foreign tissue in a mammal with a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent enhances occludin-mediated cell adhesion.

139. A method according to claim 138, wherein said modulating agent comprises at least two LYHY (SEQ ID NO:1) sequences separated by a linker.

140. A method according to claim 138, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

141. A method for enhancing adhesion of foreign tissue implanted within a mammal, comprising contacting a site of implantation of foreign tissue in a mammal with a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent enhances occludin-

mediated cell adhesion.

142. A method according to claim 138 or claim 141, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

143. A method according to claim 142, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

144. A method according to claim 138 or claim 141 wherein said modulating agent is linked to a targeting agent.

145. A method according to claim 138 or claim 141, wherein said modulating agent is linked to a support material.

146. A method according to claim 138 or claim 141 wherein said foreign tissue is a skin graft or organ implant.

147. A method according to claim 138 or claim 141, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

148. A method according to claim 147, wherein said pharmaceutical composition further comprises a modulator of cell adhesion, comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

149. A method according to claim 148, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

150. A method for inducing apoptosis in an occludin-expressing cell, comprising contacting an occludin-expressing cell with a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated cell adhesion.

151. A method according to claim 150, wherein said modulating agent comprises a sequence selected from the group consisting of QYLYHYCVVD (SEQ ID NO:2), YLYHYCVVD (SEQ ID NO:12), LYHYCVVD (SEQ ID NO:13), QYLYHYC (SEQ ID NO:14), YLYHYC (SEQ ID NO:15), LYHYC (SEQ ID NO:16), QYLYHY (SEQ ID NO:17), YLYHY (SEQ ID NO:18), CLYHYC (SEQ ID NO:3), CYLYHYC (SEQ ID NO:40), CQYLYHYC(SEQ ID NO:41), KQYLYHYD (SEQ ID NO:42), YLYHY(SEQ ID NO:43), QYLYHY (SEQ ID NO:44), KLYHYD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

152. A method for inducing apoptosis in an occludin-expressing cell,

comprising contacting an occludin-expressing cell with a cell adhesion modulating agent, wherein said modulating agent comprises an antibody or fragment thereof that specifically binds to an occludin cell adhesion recognition sequence, and wherein said modulating agent inhibits occludin-mediated cell adhesion.

153. A method according to claim 150 or claim 152, wherein said modulating agent further comprises one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin, wherein said cell adhesion recognition sequence is separated from any LYHY (SEQ ID NO:1) sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

154. A method according to claim 153, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

155. A method according to claim 150 or claim 152, wherein said modulating agent is linked to a targeting agent.

156. A method according to claim 150 or claim 152, wherein said modulating agent is linked to a drug.

157. A method according to claim 150 or claim 152, wherein said modulating agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

158. A method according to claim 157, wherein said pharmaceutical composition further comprises a modulator of cell adhesion, comprising one or more of:

(a) a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than an occludin.

159. A method according to claim 158, wherein said cell adhesion recognition sequence comprises one or more sequences selected from the group consisting of HAV, NQK, NRN, NKD, EKD, ERD, RGD, DDK, EEY, EAQ, IYSY (SEQ ID NO:49), TSSY (SEQ ID NO:50), VTAF (SEQ ID NO:51) and VSAF (SEQ ID NO:52).

160. A method for modulating the immune system of a mammal, comprising administering to a mammal a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1).

161. A method for modulating the formation of epithelial cell tight junctions, comprising administering to a mammal a cell adhesion modulating agent that comprises the sequence LYHY (SEQ ID NO:1).

162. A method according to claim 161, wherein the modulating agent stimulates the formation of epithelial cell tight junctions.

163. A method for inhibiting the development of diarrhea in a patient, comprising administering to a patient a cell adhesion modulating agent that comprises the sequence LYHY (SEQ ID NO:1), wherein said modulating agent stimulates epithelial cell adhesion.

164. A method according to claim 164, wherein the agent is administered orally.

165. A method for identifying an agent capable of modulating occludin-mediated cell adhesion, comprising:

(a) culturing cells that express an occludin in the presence and absence of a candidate agent, under conditions and for a time sufficient to allow cell adhesion; and

(b) visually evaluating the extent of cell adhesion among said cells, and therefrom identifying an agent capable of modulating occludin-mediated cell adhesion.

166. A method according to claim 165, wherein said cells are selected from the group consisting of endothelial, epithelial and cancer cells.

167. A method for identifying an agent capable of modulating occludin-mediated cell adhesion, comprising:

(a) culturing normal rat kidney cells in the presence and absence of a candidate agent, under conditions and for a time sufficient to allow cell adhesion; and

(b) comparing the level of cell surface occludin and E-cadherin for cells cultured in the presence of candidate agent to the level for cells cultured in the absence of candidate agent, and therefrom identifying an agent capable of modulating occludin-mediated cell adhesion.

168. A method for identifying an agent capable of modulating occludin-mediated cell adhesion, comprising:

(a) culturing human aortic endothelial cells in the presence and absence of a candidate agent, under conditions and for a time sufficient to allow cell adhesion; and

(b) comparing the level of cell surface occludin and VE-cadherin for cells cultured in the presence of candidate agent to the level for cells cultured in the absence of candidate agent, and therefrom identifying an agent capable of modulating occludin-mediated cell adhesion.

169. A method for detecting the presence of occludin-expressing cells in a sample, comprising:

(a) contacting a sample with an antibody that binds to an occludin comprising the sequence LYHY (SEQ ID NO:1) under conditions and for a time sufficient to allow formation of an antibody-occludin complex; and

(b) detecting the level of antibody-occludin complex, and therefrom detecting the presence of occludin-expressing cells in the sample.

170. A method according to claim 169, wherein said antibody is linked to a support material.

171. A method according to claim 169, wherein said antibody is linked to a detectable marker.

172. A method according to claim 171, wherein said detectable marker is a fluorescent marker, and wherein the step of detecting is performed using fluorescence activated cell sorting.

173. A kit for detecting the presence of occludin-expressing cells in a sample, comprising:

(a) an antibody that binds to a modulating agent comprising the sequence LYHY (SEQ ID NO:1); and

(b) a detection reagent.

174. A kit for enhancing transdermal drug delivery, comprising:

(a) a skin patch; and

(b) a cell adhesion modulating agent, wherein said modulating agent comprises the sequence LYHY (SEQ ID NO:1), and wherein said modulating agent inhibits occludin-mediated cell adhesion.

175. A method for identifying a compound capable of modulating occludin-mediated cell adhesion, comprising:

(a) contacting an antibody that binds to a modulating agent comprising the sequence LYHY (SEQ ID NO:1) with a test compound; and

(b) detecting the level of antibody that binds to the test compound, and therefrom identifying a compound capable of modulating occludin-mediated cell adhesion.

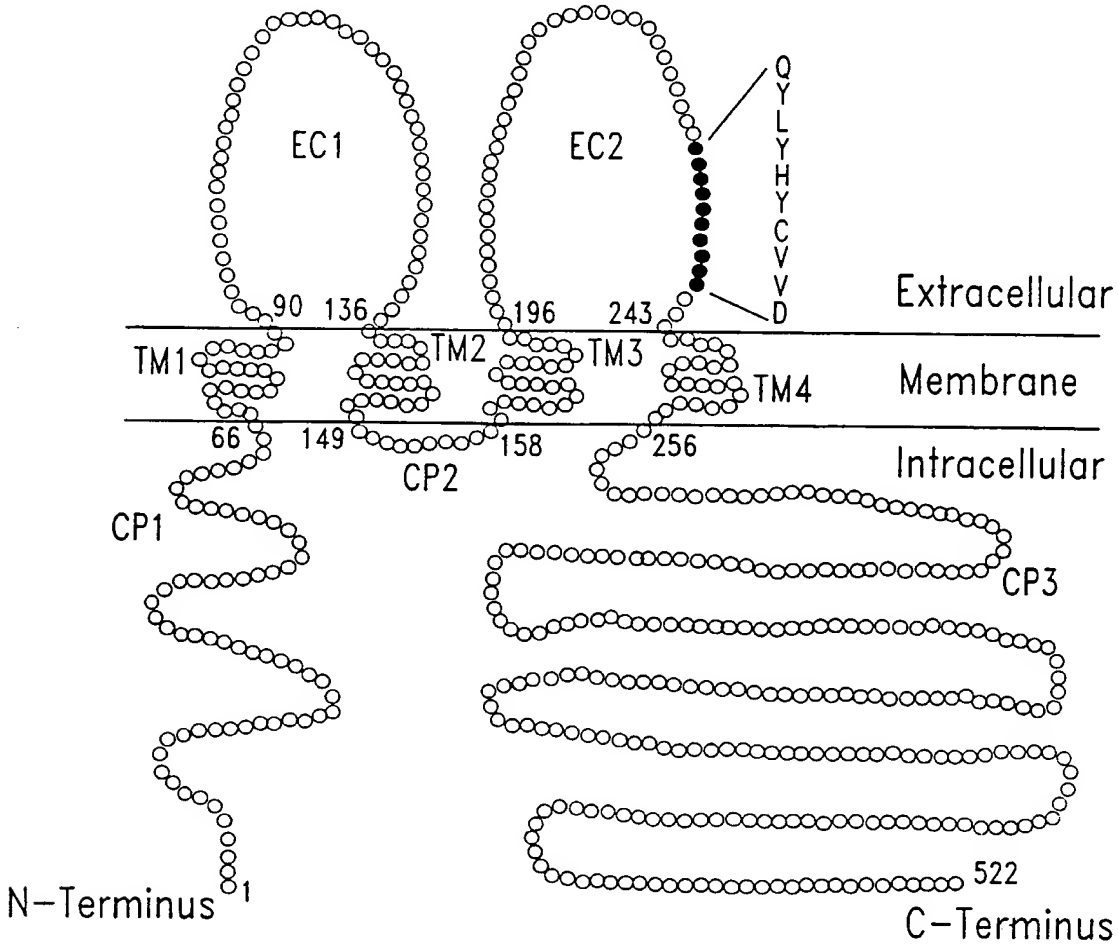
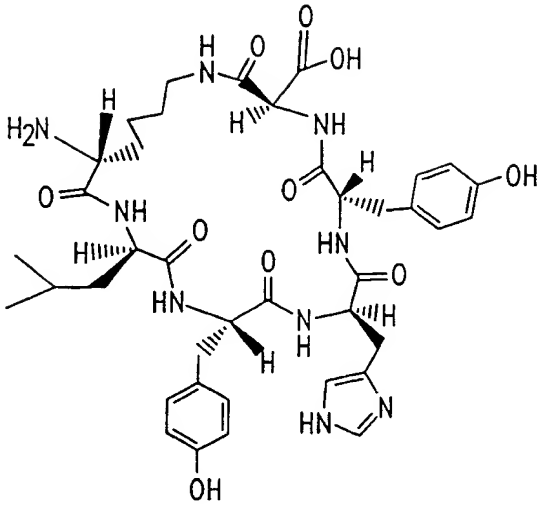

EC1
EC2
QYLYHYCVVD
Extracellular
90
136
196
243
TM1
TM2
TM3
TM4
Membrane
66
149
158
256
CP2
Intracellular
CP1
CP3
N–Terminus
1
522
C–Terminus

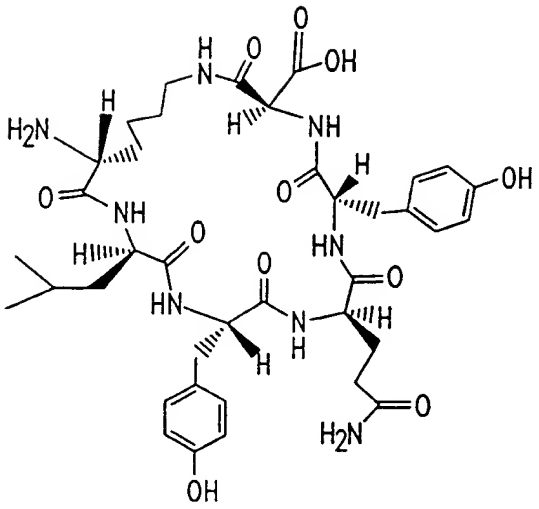
Fig. 1

```
Dog           G V N P T A Q A - - - S G S L Y S S Q I Y A M C N Q F Y A S T A T G L Y M D Q Y L Y H Y C V V D P Q E
Human         G V N P T A Q S - - - S G S L Y G S Q I Y A L C N Q F Y T P A A T G L Y V D Q Y L Y H Y C V V D P Q E
Mouse         G V N P T A Q A - - - S G S M Y G S Q I Y M I C N Q F Y T P G G T G L Y V D Q Y L Y H Y C V V D P Q E
Rat-kangaroo  G V N P R A G L G A S S G S L Y Y N Q M L M L C N Q M M S P V A G G - I M N Q Y L Y H Y C M V D P Q E
              : : : :   :           : : :   :     :         : : :               :         : : : : : : :   : : : : :
Consensus     G V N P t A q x g a s S G S l Y x s Q i y x x C N Q f y x p x a t G l y x d Q Y L Y H Y C v V D P Q E
```

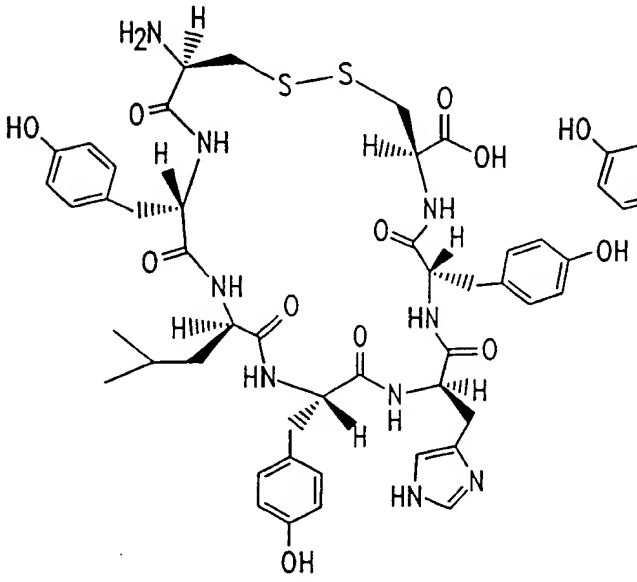
Fig. 2



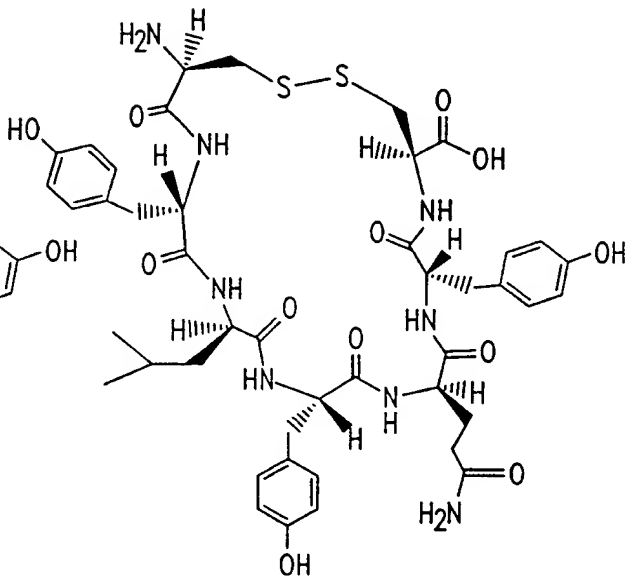
H-KLYHYD-OH



H-KLYQYD-OH

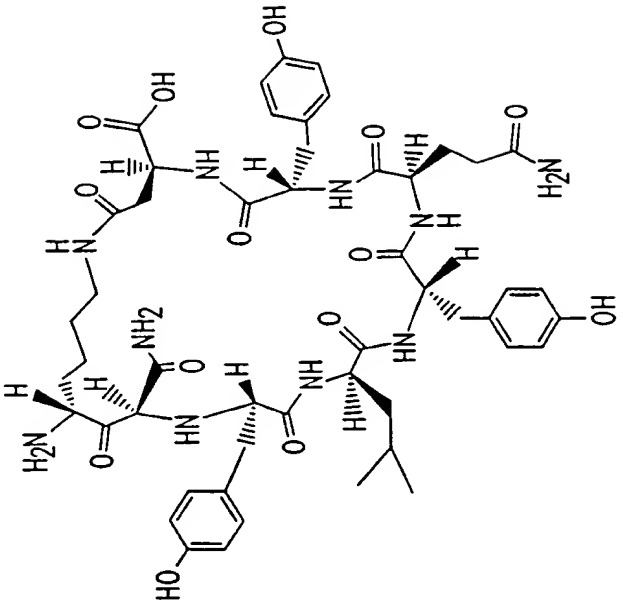


H-CYLYHYC-OH

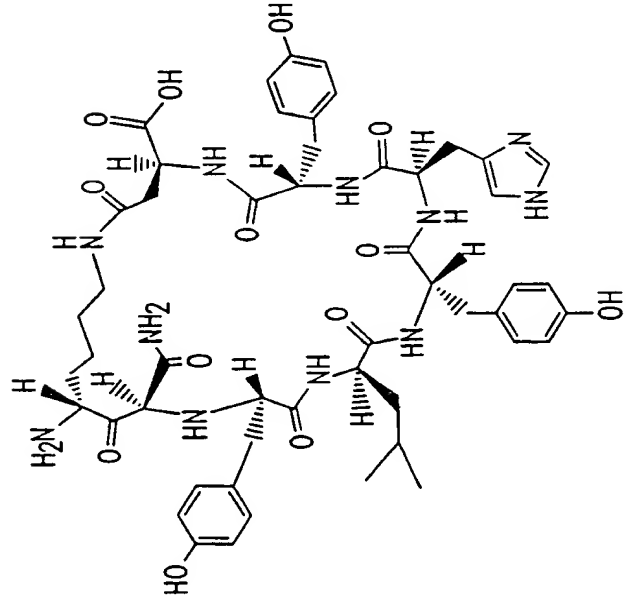


H-CYLYQYC-OH

Fig. 3A

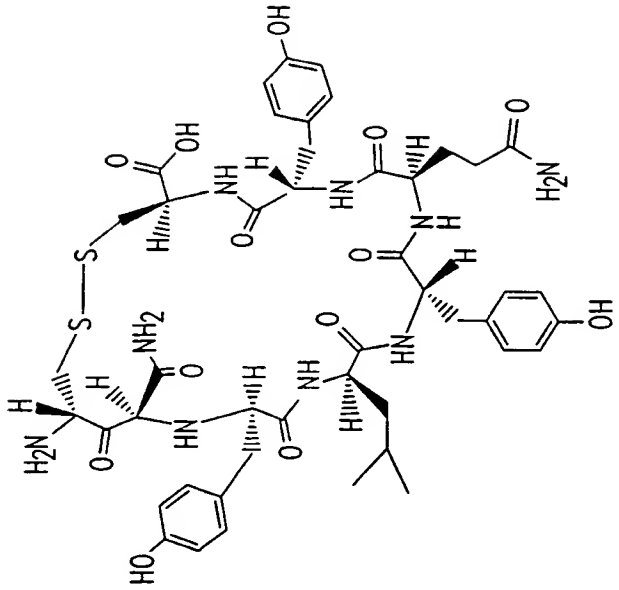


H-KQYLYQYD-OH

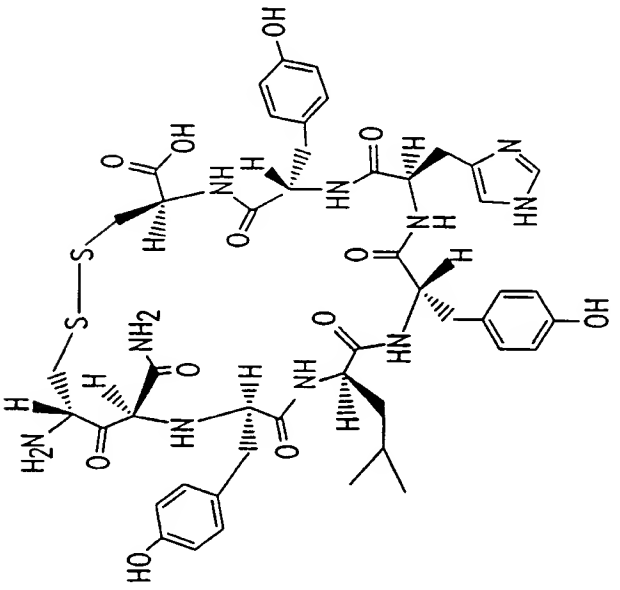


H-KQYLYHYD-OH

Fig. 3B

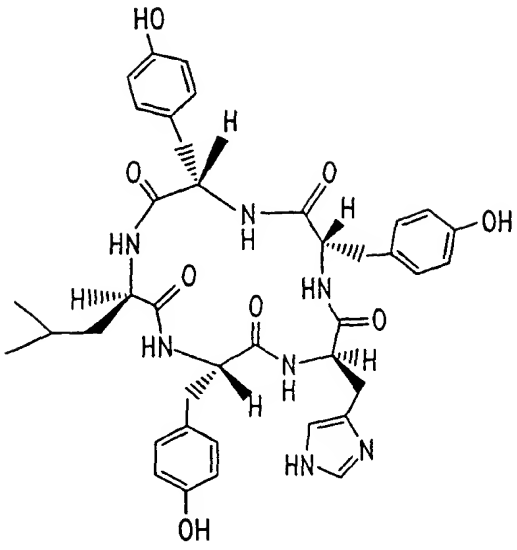


H-CQYLYQYC-OH

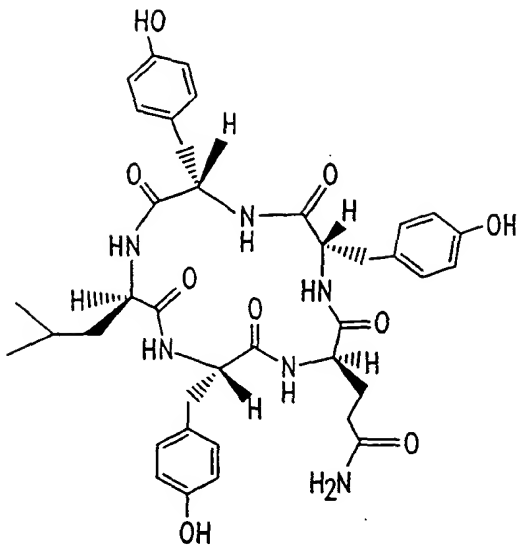


H-CQYLYHYC-OH

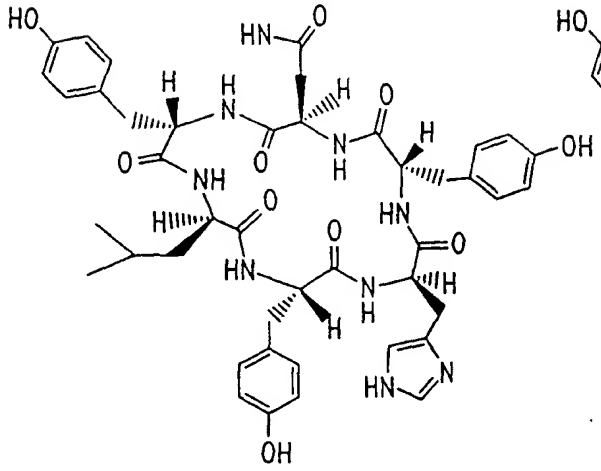
Fig. 3C



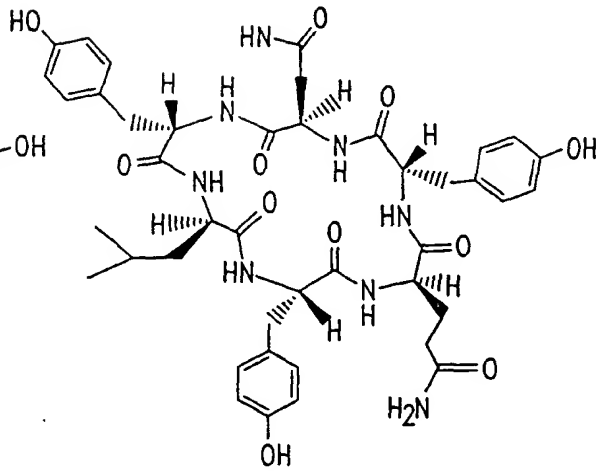
YLYHY



YLYQY

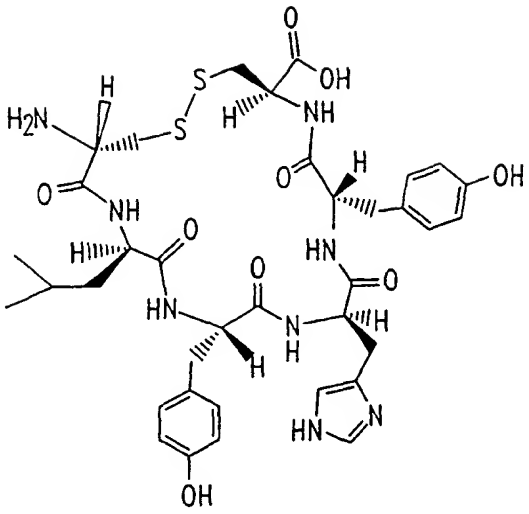


QYLYHY

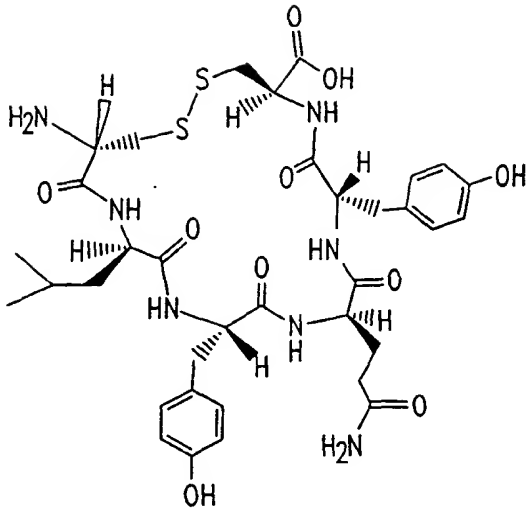


QYLYHY

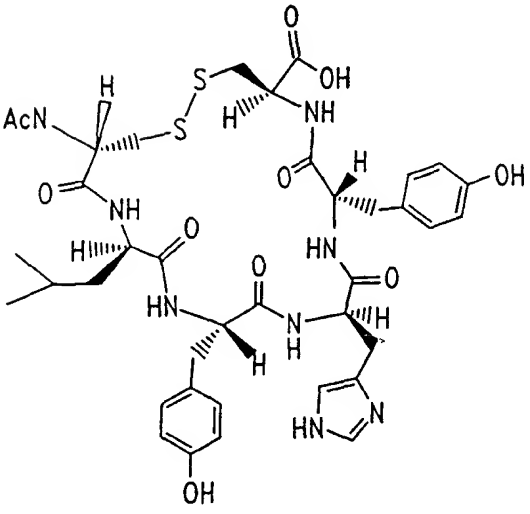
Fig. 3D



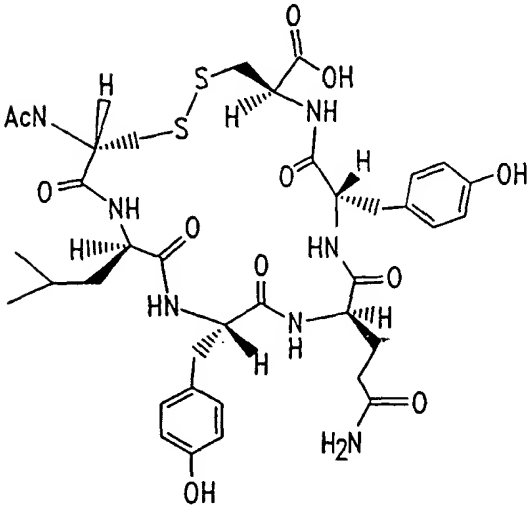
H-CLYHYC-OH



H-CLYQYC-OH



Ac-N-CLYHYC-OH



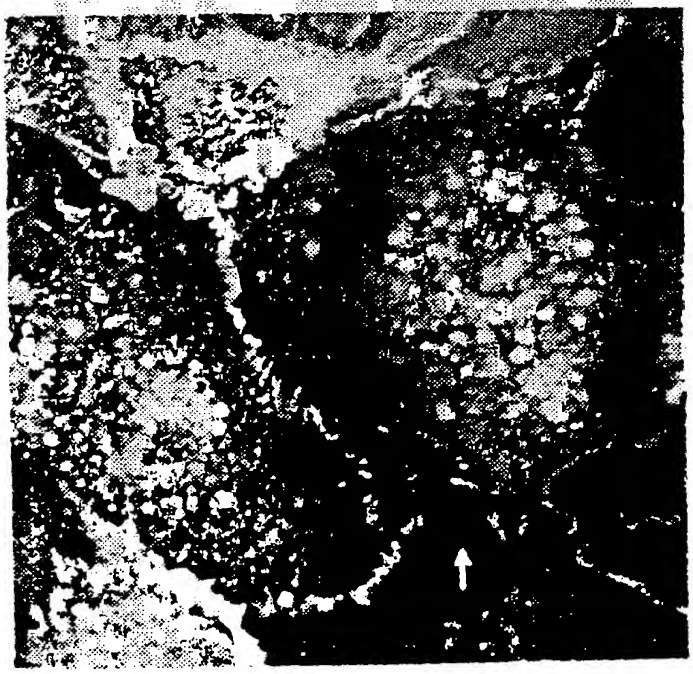
Ac-N-CLYQYC-OH

Fig. 3E

Fig. 4B

Peptide 3
(100μg/mL)

Fig. 4A



Control

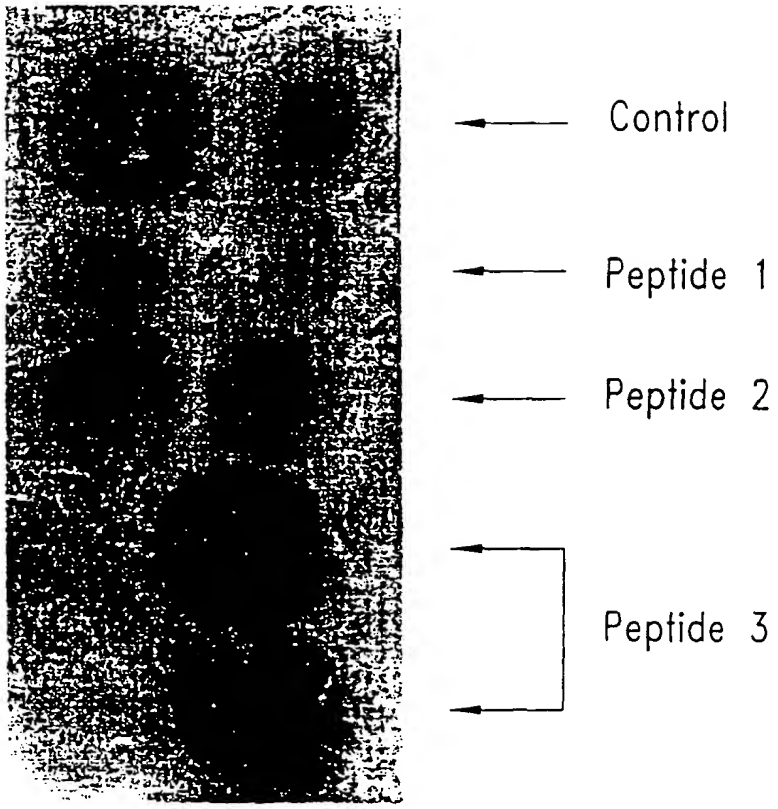


Fig. 5

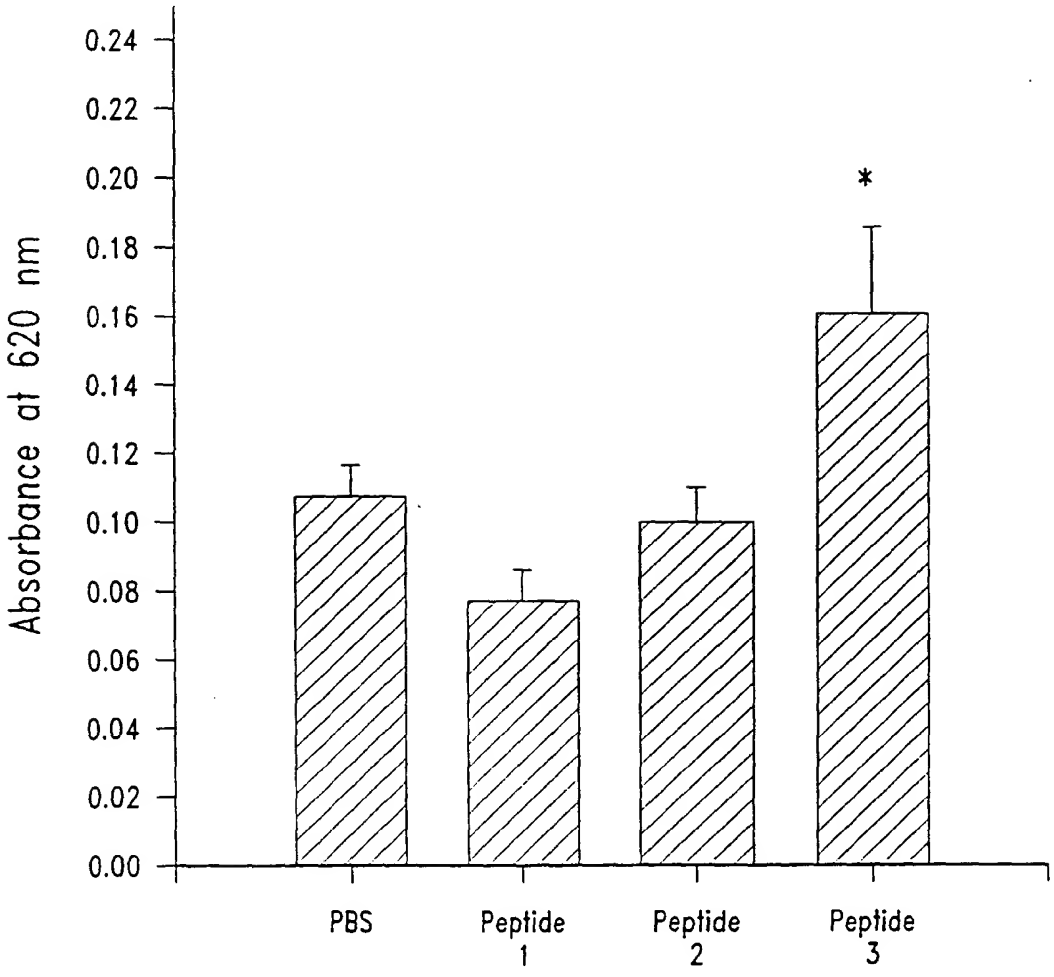

Absorbance at 620 nm
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
*
PBS
Peptide 1
Peptide 2
Peptide 3

Fig. 6

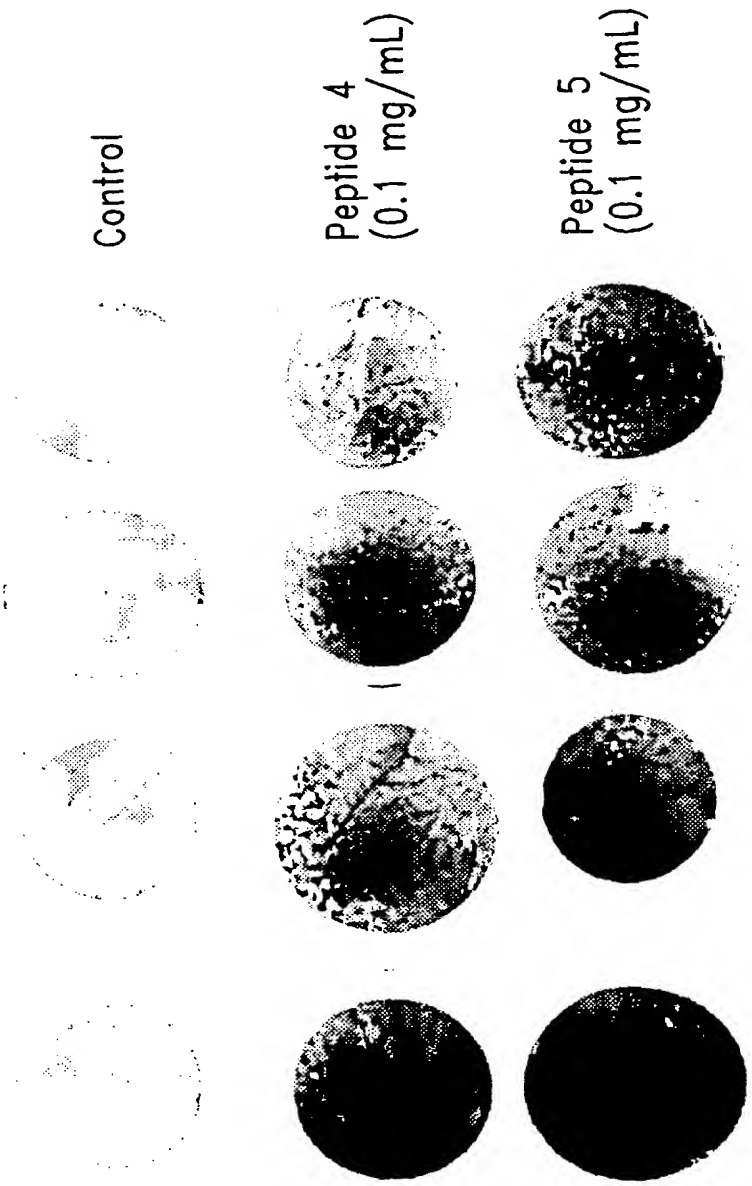


Fig. 7

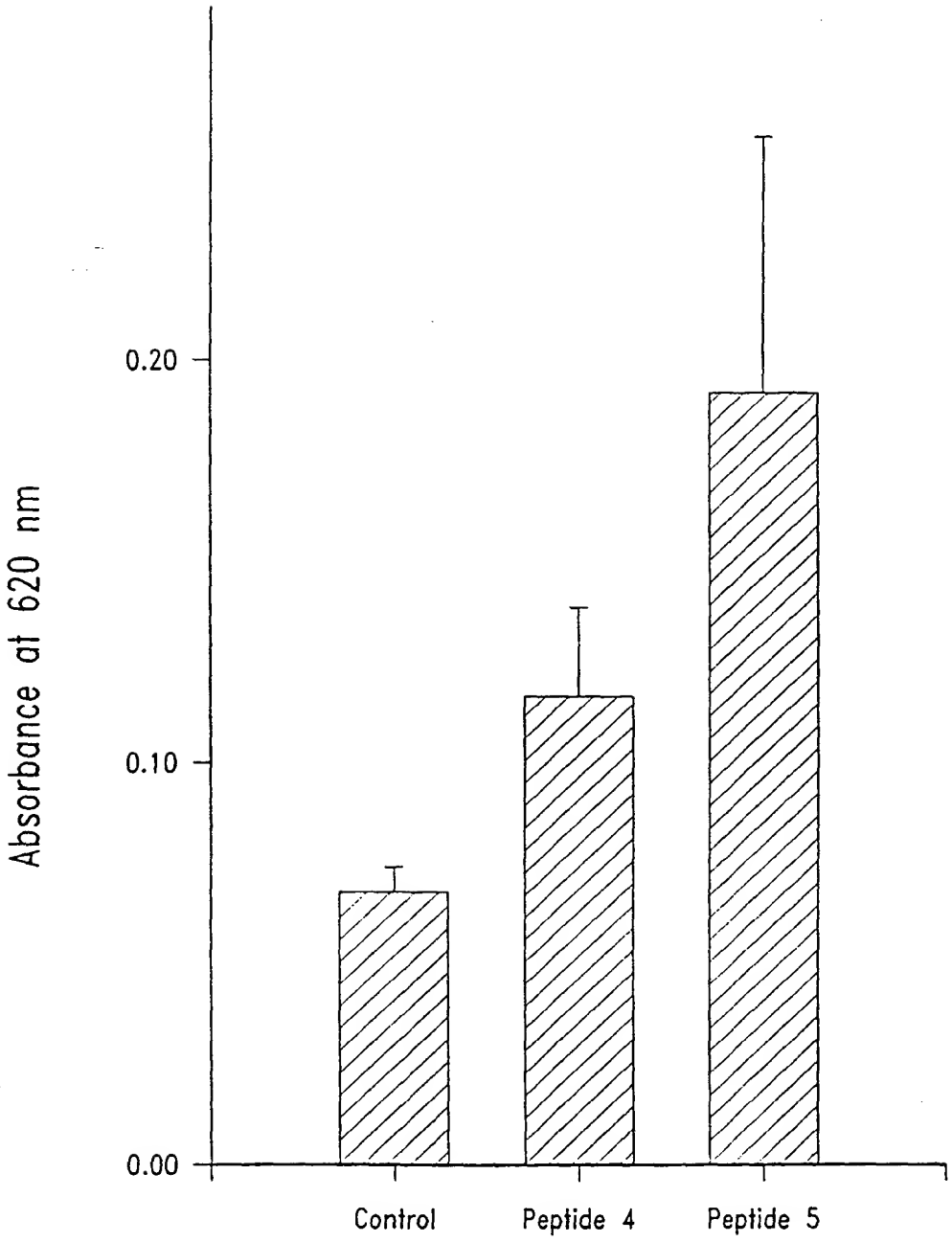


Fig. 8

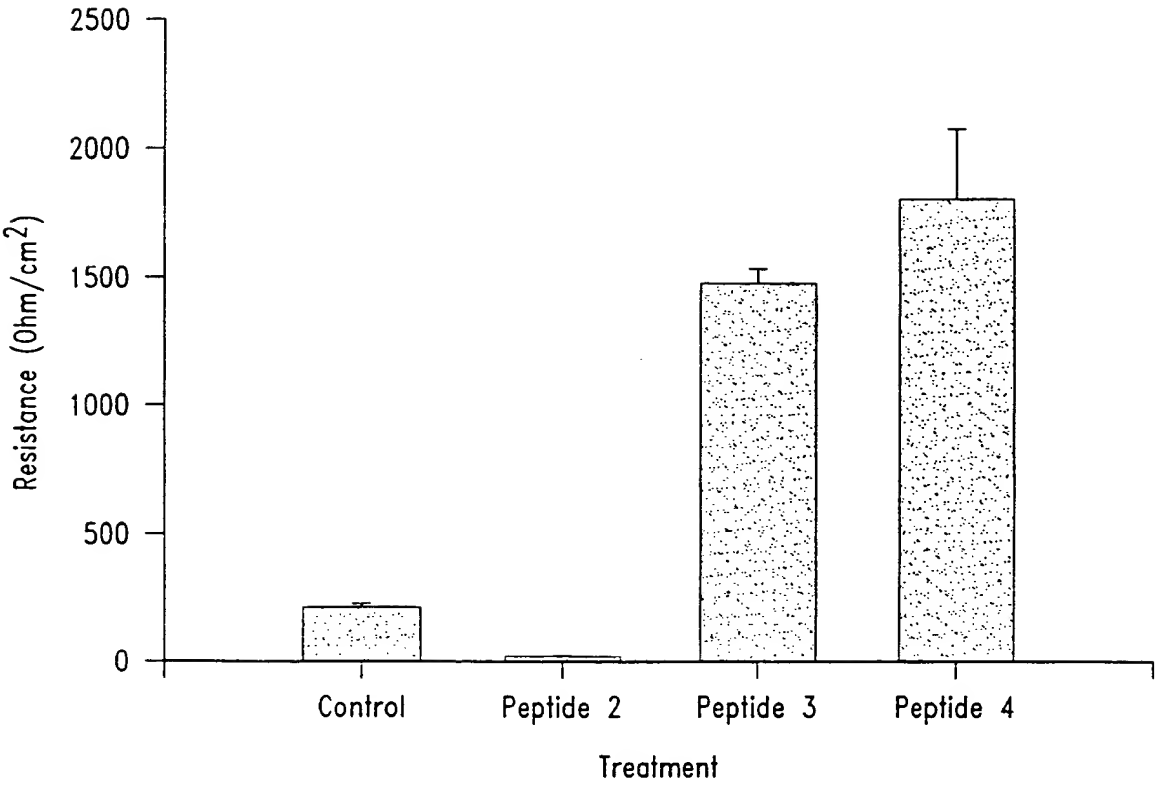

2500
2000
1500
1000
500
0
Resistance (Ohm/cm2)
Control
Peptide 2
Peptide 3
Peptide 4
Treatment

Fig. 9

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Adherex Technologies

(ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR MODULATING TISSUE PERMEABILITY

(iii) NUMBER OF SEQUENCES: 52

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Gowling, Strathy & Henderson
(B) STREET: Commerce Court West, Suite 4900
(C) CITY: Toronto
(D) STATE: Ontario
(E) COUNTRY: CANADA
(F) ZIP: M5L 1J3

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 30 December 1998
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Omar A. Nassif
(B) REGISTRATION NUMBER: 4016
(C) REFERENCE/DOCKET NUMBER: T8464447WO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (416) 862-7525
(B) TELEFAX: (416) 862-7661

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Leu Tyr His Tyr
1

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln Tyr Leu Tyr His Tyr Cys Val Val Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Phe His Leu Arg Ala His Ala Val Asp Ile Asn Gly Asn Gln Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Val Asn Pro Thr Ala Gln Ser Ser Gly Ser Leu Tyr Gly Ser Gln
1 5 10 15

Ile Tyr Ala Leu Cys Asn Gln Phe Tyr Thr Pro Ala Ala Thr Gly Leu
20 25 30

Tyr Val Asp Gln Tyr Leu Tyr His Tyr Cys Val Val Asp Pro Gln Glu
35 40 45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gly Val Asn Pro Thr Ala Gln Ala Ser Gly Ser Met Tyr Gly Ser Gln
1 5 10 15

Ile Tyr Met Ile Cys Asn Gln Phe Tyr Thr Pro Gly Gly Thr Gly Leu
20 25 30

Tyr Val Asp Gln Tyr Leu Tyr His Tyr Cys Val Val Asp Pro Gln Glu
35 40 45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Val Asn Pro Thr Ala Gln Ala Ser Gly Ser Leu Tyr Ser Ser Gln
1 5 10 15

Ile Tyr Ala Met Cys Asn Gln Phe Tyr Ala Ser Thr Ala Thr Gly Leu
20 25 30

Tyr Met Asp Gln Tyr Leu Tyr His Tyr Cys Val Val Asp Pro Gln Glu
35 40 45

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Val Asn Pro Arg Ala Gly Leu Gly Ala Ser Ser Gly Ser Leu Tyr
1 5 10 15

Tyr Asn Gln Met Leu Met Leu Cys Asn Gln Met Met Ser Pro Val Ala
20 25 30

Gly Gly Ile Met Asn Gln Tyr Leu Tyr His Tyr Cys Met Val Asp Pro
35 40 45

Gln Glu
50

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Leu Tyr His Tyr Leu Tyr His Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln Leu Tyr His Tyr Gln Leu Tyr His Tyr Gln Leu Tyr His Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Tyr Ser Phe Asn Tyr Asp Gly Ser Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Tyr Leu Tyr His Tyr Cys Val Val Asp
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Leu Tyr His Tyr Cys Val Val Asp
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gln Tyr Leu Tyr His Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Tyr Leu Tyr His Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Cys Asp Gly Tyr Pro Lys Asp Cys Lys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Cys Asp Gly Tyr Pro Lys Asp Cys Lys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /product= "OTHER"
/note= "Wherein Xaa is either S-trityl or S-acetamidomethyl"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /product= "OTHER"
/note= "Wherein Xaa is either S-trityl or S-acetamidomethyl"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Xaa Gly Asn Leu Ser Thr Cys Xaa Met Leu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Cys Gly Asn Leu Ser Thr Cys Met Leu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Cys Tyr Ile Gln Asn Cys Pro Leu Gly
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Cys Tyr Ile Gln Asn Cys Pro Leu Gly
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Cys Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Cys Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Gln Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Gln Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Cys Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6

(D) OTHER INFORMATION: /product= "OTHER"
/note= "Wherein Xaa is beta,beta-dimethyl cysteine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Leu Tyr His Tyr Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /product= "OTHER"
/note= "Wherein Xaa is beta,beta-tetramethylene cysteine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Xaa Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Xaa Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /product= "OTHER"
/note= "Wherein Xaa is beat-mercaptopropionic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Xaa Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /product= "OTHER"
/note= "Wherein Xaa is
beta,beta-pentamethylene-beta-mercaptopropionic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Xaa Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Lys Leu Tyr His Tyr Asp
1 5

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Lys Gln Tyr Leu Tyr His Tyr Asp
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Trp Gly Gly Trp
1

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Leu Phe Ser His Ala Val Ser Ser Asn Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Cys Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Cys Gln Tyr Leu Tyr His Tyr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Lys Gln Tyr Leu Tyr His Tyr Asp
1 5

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Tyr Leu Tyr His Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gln Tyr Leu Tyr His Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Lys Leu Tyr His Tyr Asp
1 5

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Gly Val Asn Pro Thr Ala Gln Xaa Gly Ala Ser Ser Gly Ser Leu Tyr
1 5 10 15

Xaa Ser Gln Ile Tyr Xaa Xaa Cys Asn Gln Phe Tyr Xaa Pro Xaa Ala
20 25 30

Thr Gly Leu Tyr Xaa Asp Gln Tyr Leu Tyr His Tyr Cys Val Val Asp
35 40 45

Pro Gln Glu
50

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Where Xaa is either Lysine or Arginine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= "Where Xaa is either Serine or Alanine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Where Xaa is either tyrosine or phenylalanine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Trp Xaa Xaa Xaa Xaa Xaa Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Where Xaa is isoleucine, leucine or valine"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= "Where Xaa is aspartic acid, asparigine or glutamic acid"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= "Where Xaa is serine, threonine or asparagine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ile Tyr Ser Tyr
1

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Thr Ser Ser Tyr
1

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Val Thr Ala Phe
1

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Val Ser Ala Phe
1

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/CA 98/01208

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/47 C07K14/705 C07K16/18 C07K16/28 A61K38/17
A61K39/395 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 33605 A (ITALLIE CHRISTINA M VAN ;UNIV YALE (US); ANDERSON JAMES M (US)) 18 September 1997 see the whole document ---	1,18-175
X	WONG ET AL: "A synthetic peptide corresponding to the extracellular domain of occludin perturbs the tight junction permeability barrier" THE JOURNAL OF CELL BIOLOGY, vol. 136, no. 2, 27 January 1997, pages 399-409, XP002095630 see the whole document --- -/--	1,18, 32-34, 43,48, 49,55, 59,60, 66,161, 162, 165-173, 175

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

' Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 1999	22/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int .ional Application No
PCT/CA 98/01208

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category ˇ	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
X	PIQUE C ET AL: "Among all human T-cell leukemia virus type 1 proteins, tax, polymerase, and envelope proteins are predicted as preferential targets for the HLA-A2-restricted cytotoxic T-cell response." JOURNAL OF VIROLOGY, (1996 AUG) 70 (8) 4919-26. JOURNAL CODE: KCV. ISSN: 0022-538X., XP002099340 United States see table 1 ---	32
A	FURUSE M ET AL: "OCCLUDIN: A NOVEL INTEGRAL MEMBRANE PROTEIN LOCALIZING AT TIGHT JUNCTIONS" JOURNAL OF CELL BIOLOGY, vol. 123, no. 6, 1993, pages 1777-1788, XP002060057 cited in the application see the whole document ---	
A	JAEGER E.A.: "Small synthetic peptides homologous to segments of occludin impair tight junction resealing in a Ca2+-switch assay in A6 cell monolayers" MOL.BIOL.CELL (SUPPL), vol. 8, November 1997, page 205A XP002099341 Abstract no. 1189 ---	
P,X	WO 98 21237 A (PRAECIS PHARM INC ;CHAI LING (US); HUNDAL ARVIND (US); LU KUANGHUI) 22 May 1998 See page 1, lines 16-18; page 3, lines 1-8;page 13, lines 3-11; tables 4-6;example 7; claims 1-3,15-24 ---	32,43, 98,100, 105,108, 114,127, 135
P,X	EP 0 831 148 A (EISAI CO LTD) 25 March 1998 see claims 16,21 -----	34

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

..ernational application No.
PCT/CA 98/01208

Box I Observations where certain claims w re found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 48-164 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No
PCT/CA 98/01208

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9733605 A	18-09-1997	NONE	
WO 9821237 A	22-05-1998	AU 5357798 A	03-06-1998
EP 0831148 A	25-03-1998	WO 9732982 A	12-09-1997